Exercise to boost the immune system and optimise immunotherapy responsiveness

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A high proportion of this thesis has been published. The introduction is a published article review (Chapter 1). The study protocol has been registered with clinicaltrials.gov and therefore in the public domain (Chapter 5). My confirmation review/ literature review from Year 1 can largely be found within Chapters 1, 2, 3 & 5.

Publications

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Prizes

1) First place best oral presentation at the UoS SMPH Annual Research & Innovation Meeting, June 2024
2) PGR recognition award from SMPH, February 2025

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Abbreviations

μl - microlitres
4T1 – Mouse triple negative breast cancer cell line
A549 – Human lung cancer cell line
AB-1 – Mouse mesothelioma cell line
AE – Adverse event
AF – Alexa Fluor
AMPK - AMP-activated Protein Kinase
ANGPT2 – Angiopoietin 2 (angiogenic gene)
APC – Allophycocyanin
APR – Annual progress report
ASCO – American Society of Clinical Oncology
AWRC - Advanced Wellbeing Research Centre
B16F10 – Melanoma cell line
BALB/c – Inbred albino mouse stain
BD – Bectin Dickinson
BICEP - Exercise to Boost Immunity in advanced Cancer: feasibility of combined aerobic Exercise and resistance training for Patients with advanced mesothelioma and pancreatic cancer
BV – Brilliant violet
C57BL/6 – Inbred mouse strain
CCL - C-C motif ligand
CI – Chief Investigator
CO2 - Carbon dioxide
Colon-26 – Mouse colorectal cancer cell line
CRE - Clinical Radiation Expert
CRFs – Case report forms
CRIO - Clinical Research and Innovation Office
CT- Computed Tomography
CTIMP - Clinical Trial of an Investigational Medicinal Product
CTLA-4 - Cytotoxic T lymphocyte antigen 4
CXCL – Chemokine ligand
CXCR – Chemokine receptor
CXCR2 – Myeloid cell receptor
DAPI – 4',6-diamidino-2-phenylindole
DMEM - Dulbecco's Modified Eagle Medium
DNA - deoxyribonucleic acid
DNA – Deoxyribonucleic Acid
DPX - Distyrene, Plasticizer, and Xylene
DU145 – Prostate cancer cell line
ECOG – Eastern Cooperative Oncology Group
EDTA - Ethylenediaminetetraacetic acid
ELISA - Enzyme-linked immunosorbent assay
EMT - Epithelial-mesenchymal transition
EO771 – Human breast cancer cell line
EQ-5d – Standardised quality of life questionnaire
F - Fungizone
FACS - Fluorescence-activated Cell Sorting
FBS - Foetal bovine serum

FITC - Fluorescein Isothiocyanate
FITT – Frequency, Intensity, Time and Type
FMO - Fluorescence Minus One
FOLFIRINOX – 5-Fluorouracil, irinotecan, oxaliplatin
GCP – Good Clinical Practice
GFP – Green Fluorescent Protein
GrM1- Glutamate receptor
GzmB – Granzyme B
H&E - Haematoxylin and Eosin
HER2 – Human Epidermal Growth Factor Receptor 2
HIF-1 - Hypoxia-inducible factor-1
HLA-A2+ - Human leucocyte antigen-A2-positive
HR – Heart rate
HRA - Health Research Authority
HSV – Herpes simplex virus
IC50 – Half maximal inhibitory concentration
ICIs- Immune checkpoint inhibitors
IFN-γ - Interferon gamma
IL – Interleukin
IMS - Industrial Methylated Spirits
iNOS – inducible Nitric Oxide Synthase
IRAS - Integrated Research Application System
IRAS - Integrated Research Application System
IVIS – In Vivo Imaging System
JJN3 – Human myeloma cell line
Ki-67 – Marker of cell proliferation
KPC – Mouse pancreatic ductal adenocarcinoma cell line
KRAS – Kirsten Rat Sarcoma viral oncogene
LoVo – Human colorectal cancer cell line
MDSCs - Myeloid-derived Suppressor Cells
MHC – Major Histocompatibility Complex
MHC – Major histocompatibility complex
ml - millilitres
MLTL – More Life to Live
MMP - Matrix metalloproteinases
MOI - Multiplicity of Infection
MPE - Medical Physics Expert
MRC – Medical Research Council
MSTO-211H – Human mesothelioma cell line
mTOR - mammalian Target of Rapamycin
MTRG - Mandard Tumour Regression Grade
NCR1 - Natural cytotoxicity triggering receptor 1
NHS – National Health Service
NIHR - National Institute for Health and Care Research
NIR – Near-Infrared
NK cells – Natural Killer cells
NKG2D – Natural Killer cell activating receptor
NKp46 - Natural Killer cell activating receptor
NMRI - Naval Medical Research Institute mice

NOD SCID γ mice - Nonobese Diabetic/ Severe Combined Immunodeficient gamma mice
NSCLC – Non-Small Cell Lung Cancer
OSM - Oncostatin M
OV – Oncolytic virus
P/S - Penicillin-streptomycin
p/s – photons per second
Panc02 – Mouse pancreatic cancer cell line
PANC-1 – Human pancreatic cancer cell line
PARP - poly (ADP-ribose) polymerase
PBMC – Peripheral blood mononuclear cell
PBS – Phosphate buffered saline
PBST – Phosphate buffered saline - Tween20
PCUK – Pancreatic Cancer UK
PD-1 – Programmed Cell Death Protein 1
PDA - Pancreatic Ductal Adenocarcinoma
PDL-1 – Programmed Cell Death Ligand 1
PE – Phycoerythrin
PerCP/CY5.5 - Peridinin chlorophyll protein-Cyanine 5.5
PFA – Paraformaldehyde
PFN – Perforin,
PFS – Progression-free survival
pfu – plaque forming units
pg – picograms
PI – Principal Investigator
PIS – Patient information sheet
PPI – Patient and Public Involvement
REC - Research Ethics Committee
RECIST 1.1 – Radiology reporting criteria when measuring tumours on imaging
RfPB – Research for Patient Benefit funding scheme
RIF – Research Innovation Fund
RM – Repetition Maximum
RNA – Ribonucleic Acid
ROUT – Robust Outlier Test
RPE – Rate of Perceived Exertion
RPM – Revolutions per Minute
RPMI - Roswell Park Memorial Institute medium
SAE – Severe adverse event
SD – Standard deviation
SHU – Sheffield Hallam University
SOPs – Standard Operating Procedures
SPARC - Secreted Protein Acidic and Rich in Cysteine
STH - Sheffield Teaching Hospitals NHS Foundation Trust
SURE - Sheffield Undergraduate Research Experience
TAMs- Tumour associated macrophages
TCR – T cell Receptor
TGFβ - Transforming growth factor beta
TME- Tumour microenvironment
TNFα – Tumour Necrosis Factor alpha
Tp53 – Tumour suppressor gene

TRAMP C-1 - Transgenic Adenocarcinoma of Mouse Prostate C-1 cells
Tregs – T regulatory cells
T-VEC - Talimogene Laherparepvec
UoS – University of Sheffield
VEGF-α - Vascular endothelial growth factor alpha
VO2 max – maximum rate of oxygen consumption
WB-EMS - Whole body electro-myosimulation
YCR – Yorkshire Cancer Research
γ-H2AX – Phosphorylated histone H2AX

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All schematic figures were created using BioRender.com

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Abstract

Background:

Exercise is known to modulate the immune system, supporting anti-cancer activity. Pancreatic cancer and mesothelioma derive limited benefit from immunotherapy due to their immune-deplete tumour microenvironments (TME). We hypothesised that exercise could enhance immune function and improve immunotherapy responsiveness in these cancers.

Methodology:

Healthy volunteers (n=22) completed a high-intensity cycling session with blood collected pre- and post-exercise. Flow cytometry was used to characterise leucocyte subpopulations, and pancreatic cancer and mesothelioma cell viability, migration, and apoptosis were assessed using pre- and post-exercise serum.

In vivo, C57BL/6 (n=20) and BALB/c (n=24) mice were orthotopically implanted with pancreatic cancer and mesothelioma, respectively. Mice underwent treadmill running and resistance training with anti-Programmed Cell Death Protein 1 (PD1), compared with no exercise and isotype controls. TME immune populations were analysed by flow cytometry and immunofluorescence. Tumour and liver tissues were assessed for necrosis and metastases.

Results:

In healthy volunteers, exercise increased Natural Killer (NK) cells ($p<0.0001$) and reduced regulatory T cells (Tregs) ($p<0.0001$). Exercise-conditioned serum decreased cancer cell migration (pancreatic $p=0.0313$; mesothelioma $p=0.0249$) and increased apoptosis (pancreatic $p=0.0099$; mesothelioma $p=0.0313$).

In mice, exercise/anti-PD1 was feasible from Day 2 post-implantation. In pancreatic cancer, exercise reduced myeloid derived suppressor cells (MDSCs) and Tregs in the TME ($p=0.0426$ and $p=0.0115$, respectively), decreased liver metastases, and increased tumour necrosis ($p=0.0286$ and $p=0.0244$). Exercise/anti-PD1 also enhanced CD8+PD1+ T cell infiltration in both pancreatic cancer and mesothelioma ($p=0.0247$ and $p=0.0316$, respectively).

Conclusions:

Acute aerobic exercise in healthy volunteers promoted systemic cytotoxicity (NK cell expansion) and reduced immune tolerance (Treg suppression). Exercise-conditioned serum exerted direct anti-tumour effects on pancreatic cancer and mesothelioma cells. In mouse models, reduced-intensity exercise elicited anti-tumour activity, and exercise combined with anti-PD1 improved tumour control and reduced metastases in pancreatic cancer. These findings demonstrate the potential of exercise to enhance immunotherapy efficacy across cancer types.

Chapter 1

Introduction

Please note the introduction has already been published ([Frontiers | Exercise to transform tumours from cold to hot and improve immunotherapy responsiveness]**). There are minor modifications to incorporate the breadth of this research project.**

Introduction

1.1: The immune climate of the tumour microenvironment: cold vs hot

Immunotherapies, such as immune checkpoint inhibitors (ICIs), have significantly extended survival outcomes across multiple different cancers. Immune system evasion is a key tactic utilised by cancer cells to allow their growth, migration and invasion. ICIs facilitate cancer cell recognition and immune cell destruction by inhibiting pathways they use to evade the immune system. Although ICIs have demonstrated significant benefits in cancer care, they are limited by a spectrum of potentially serious side-effects, including fatigue and immune-mediated toxicities such as hepatitis, colitis and pneumonitis (1). Fewer than 40% of patients derive significant benefit from ICIs which highlights the need to better understand the mechanisms for resistance to immunotherapy and how to overcome this to improve responsiveness (1,2). ICI response correlates with the degree of immune cell infiltration within the tumour microenvironment (TME), which varies significantly between tumours on the cold to hot spectrum.

When a TME lacks effector immune cells such as CD8+ T cells and Natural Killer (NK) cells, as in the cases of colorectal and pancreatic cancer, this is considered a 'cold tumour' (3). Myeloid derived suppressor cells (MDSCs) and tumour associated macrophages (TAMs) can be found in abundance within cold tumours creating an immunosuppressive environment and consequently, tumours are able to propagate without significant resistance from the host immune system (3–5). This less favourable immune profile correlates to a limited, if any, response to ICIs (4,5).

1.2: Pancreatic cancer and mesothelioma: clinical management

Pancreatic cancer is the 10^{th} most common cancer in the UK and is particularly hard to treat with a poor prognosis and a 5-year survival rate of less than 7% (6). Pancreatic cancer is a 'cold tumour' due to the lack of immune cells within the TME and a less favourable immune profile and demonstrates limited response to anti-cancer treatment (4,5). ICIs have not demonstrated effectiveness in pancreatic cancer and the prognosis of metastatic pancreatic cancer remains poor with a median overall survival of 11.1 months with optimal chemotherapy (7). Only 30% of patients obtain a radiological response with first line palliative combination chemotherapy FOLFIRINOX (5-Fluorouracil, irinotecan and oxaliplatin) (7). Furthermore, fatigue, neutropenic sepsis, weight loss and other chemotherapy-mediated toxicities significantly reduce the quality of life of the months they have remaining.

In comparison, ‘hot’ tumours, such as melanoma, are highly immunogenic with a rich CD8+ T cell population within their TME (5). Therefore, hot tumours hold the potential for immune-mediated recognition and destruction of tumour cells, which cold tumours inherently lack. The abundance and potential anti-cancer activity of these immune components can vary between different hot tumours, partly explaining the varying responses to ICIs amongst hot tumours. Dual ICIs with nivolumab and ipilumumab have some success in the treatment of advanced mesothelioma although only 40% respond with a median overall survival of 18.1 months (8). This is a relatively modest benefit when compared to ICI response to a classically hot tumour such as melanoma, where the overall survival exceeds 60 months with dual ICIs (9). The TME in mesothelioma is complex with NK cell, CD4+ and CD8+ T cell infiltration supporting its classification as a hot tumour, however functional aberrations prevent their full utility (10). This coupled with high concentrations of pro-tumour TAMs suppress the degree of responsiveness seen with ICIs (10). Therefore, mesothelioma more likely lies in the middle of the cold-hot spectrum and shifting towards a ‘hot’ immune climate may improve the effectiveness of ICIs.

There is a growing body of evidence that exercise training can modulate local tumour and systemic immunity in patients with cancer, skewing the immune profile to favour anti-tumour activity. There is a clear unmet need in patients with advanced pancreatic cancer and mesothelioma, to improve overall response and survival outcomes as well as quality of life. Exercise training would be a simple, safe, cost-effective method to help shift tumours towards the hot end of the spectrum and to support ICIs’ mechanism of action. This would lead to improved responses and survival outcomes in patients with hot tumours undergoing immunotherapy, as well as expanding the range of cancers that can obtain benefit from immunotherapy, such as pancreatic cancer.

Furthermore, exercise has known health benefits including enhancing quality of life and ameliorating adverse effects associated with cancer and its treatment (2,3). The evidence for the effect exercise exerts on different tumour types within the cold-hot spectrum via different mechanisms is described in detail below.

1.3: Exercise and cancer

The definition of physical activity is "*any bodily movement produced by skeletal muscles or that requires contraction of your muscles and energy expenditure*" (11). In order to be classed as exercise, activity would need to consist of organised, repeated movements which when done regularly, can have a positive impact on cardiovascular and respiratory function, physical fitness and general overall health (11).
Exercise has been shown to have health benefits in the general population, both physically by improving cardiovascular, respiratory and musculoskeletal functioning and mentally (11). The National Health Service (NHS) guidelines recommend regular aerobic exercise (150 minutes of moderate intensity or 75 minutes of high intensity per week) and resistance training twice weekly (12). These recommendations are provided for the general population, however there is currently no tailored exercise advice to patients with advanced cancer. Given the wide range of functional abilities and background health conditions, a 'one size fits all' exercise regime would not be appropriate.

Evidence shows that the added benefits in patients with cancer include improving overall survival, reducing cancer-related and treatment induced fatigue along with reducing cancer recurrence (4,13). It would be prudent to define the exercise event to provide a reproducible, standardised intervention in cancer care (4). Acute exercise includes independent exercise activities compared to regularly repeated exercise lasting months with exercise training or more than a year with chronic exercise (4). Studies mainly correlate exercise training at moderate intensity aerobic exercise with the physical and mental health benefits seen in cancer patients (14,15). Furthermore, the FITT criteria (Frequency, Intensity, Time and Type) would provide information on dosing required to exhibit intended benefits and does require further research in order to cater to the advanced cancer population (4).

Cold tumours

Colorectal cancer is classically a cold tumour when it possesses microsatellite stability rendering ICIs ineffective (16). There is strong evidence that exercise has a protective role against colorectal cancer with an estimated relative risk reduction in colorectal cancer development of 12 to 28% (17). One of the proposed mechanisms behind this is the exercise-induced reduction in adipose tissue and the metabolic benefits including optimising insulin sensitivity, although studies suggest other pathways contribute to the reduced cancer risk (17). Although the indirect mechanisms described above are well documented, the direct anti-cancer processes induced by exercise require further exploration.

Zylstra et al. reviewed the effect of incorporating combined moderate intensity supervised and home-based aerobic exercise activities into the pre-operative treatment pathway, consisting of multi-agent neoadjuvant chemotherapy, for potentially resectable oesophageal cancer, which is a cold tumour (14). This single centre controlled study showed that the exercise cohort had a significantly enhanced tumour response to treatment compared to controls (14). This was demonstrated by a higher rate of Mandard Tumour Regression Grades (MTRG) of 1-2 (14). MTRG is a scoring system from 1-5, where a score of 1 would indicate no evidence of active cancer cells (complete response) and a score of 5 suggests no evidence of cancer regression (no response) within the pathology specimen (14). There are multiple limitations including non-randomisation due to geographical issues in delivering supervised exercise activities, small sample size and the modification in clinical guidelines during the study with regards to optimum chemotherapy regimen (14). Given the relatively poor prognosis of oesophageal cancer and limited treatment options, a robust randomised controlled trial is required to support exercise as an effective anti-cancer intervention and determine the effect on more relevant clinical endpoints such as overall survival.

Hot tumours

Exercise significantly inhibits tumour development and growth in preclinical models of hot tumours including transplanted Lewis lung cancer, diethylnitrosamine induced liver cancer and melanoma (GrM1) mouse models (18). Pedersen et al. (13) demonstrated anti-cancer benefits in female mice that performed exercise training over four weeks. This involved wheel running at an average of 4.1km a day for each mouse, before both subcutaneous implantation and intravenous administration of B16F10 melanoma cells

(13). A statistically significant 61% reduction in tumour size and lower incidence of metastatic lung disease in the exercised mice were seen (13). Only female mice were inoculated with this model introducing an inherent gender bias. Although the positive effect of wheel running on cancer regression was also demonstrated in five different murine models including diethylnitrosamine induced liver cancer in male Naval Medical Research Institute (NMRI) mice (13). To further explore the mechanism behind the inhibited tumour growth in the B16F10 melanoma model, microarray analysis confirmed increase in immune cell activity with enhanced gene activation, cytokine expression and immune cells both of pro-inflammatory and anti-inflammatory nature (13). Chronic inflammation correlating with increased expression of pro-inflammatory immune components can be associated with the carcinogenic process. Microarray analysis specifically noted increased interleukin (IL)-1α and inducible nitric oxide synthase (iNOS levels) (13), both involved in signalling pathways that promote tumour growth and a raised IL-1α level was shown to be a poor prognostic factor in gastric cancer and squamous cell carcinoma affecting the head and neck (19,20). Despite the upregulation of these pathways, an overall reduction in tumour growth and incidence suggests exercise induces a series of complex immune processes, which favour anti-tumour activity.

1.4: Exercise and immunity

Evidence is increasing in support of the role of exercise in modulating the immune system and TME through multiple mechanisms in cancer patients (4). ‘Exercise-induced leucocytosis’ refers to the immediate increase in circulatory leukocytes after a single exercise activity (21). Murine models have demonstrated that exercise up-regulates immune pathways in tumours including natural killer (NK) cells, B cells, T cells and dendritic cells (18). NK cell circulatory release appears to be the most sensitive and have an immediate response to acute exercise driven by catecholamine release (18). NKG2D and NKp46 are NK cell activating receptors that are upregulated within the TME with exercise (22) . Within the hour after exercise cessation, T cells continue to produce cytokines and in vitro studies have shown NK cells are more efficiently cytotoxic against myeloma and lymphoma cell lines (4,23). Improved outcomes can be seen across many different cancers with an NK cell rich TME likely due to their cytotoxic function (22).

Pederson et al. were able to show in the B16F10 melanoma murine model, lower tumour burden was associated with raised NK cell tumour infiltration (13). They were able to demonstrate tumour specificity of NK cell concentration as opposed to lymphoid organ NK cell accumulation seen in exercised control mice (13). This highlights that exercise-induced immune responses can be directed to local tumour immunity rather than a non-specific generalised mechanism and help to create a hot TME.

Despite this well evidenced exercise driven leucocytosis, the converse depletion of NK cells and CD8 T cells below baseline levels is observed after three hours of exercise completion (24). However, this lymphopaenia may reflect their redistribution from circulation to peripheral tissue supporting a continued enhancement of immune function (24).

Importantly, these studies showing correlation between exercise and increasing immune cell tumour infiltration in murine models have mainly been demonstrated in hot tumours with TMEs rich in tumour inhibiting T cells and cytokines such as interferon gamma (IFN-γ) (5) and therefore, inherently immunogenic. Multiple diverse mechanisms can result in systemic immune dysfunction and cold tumours such as pancreatic and prostate cancer, have been shown to have low immune cell populations and reduced immune activity within their TMEs as demonstrated in Figure 1.1 (4,5).

Cancer cells are able to survive and proliferate by hiding from the immune system and preventing destruction by a number of different mechanisms (22). One of these mechanisms seen in pancreatic ductal adenocarcinoma (PDA), the most common subtype of pancreatic cancer, is reconfiguring immune cell production with consequent proliferation of myeloid cells, with a preference towards an immature subset and a reduction in lymphoid cells that exhibit anti-cancer activity, subsequently promoting an immunosuppressive environment (25). The paucity of anti-tumour immune cell concentrations in PDA and consequent lack of responsiveness to immunotherapies, highlight the specific need to optimise local tumour and systemic immunity in cold tumours.

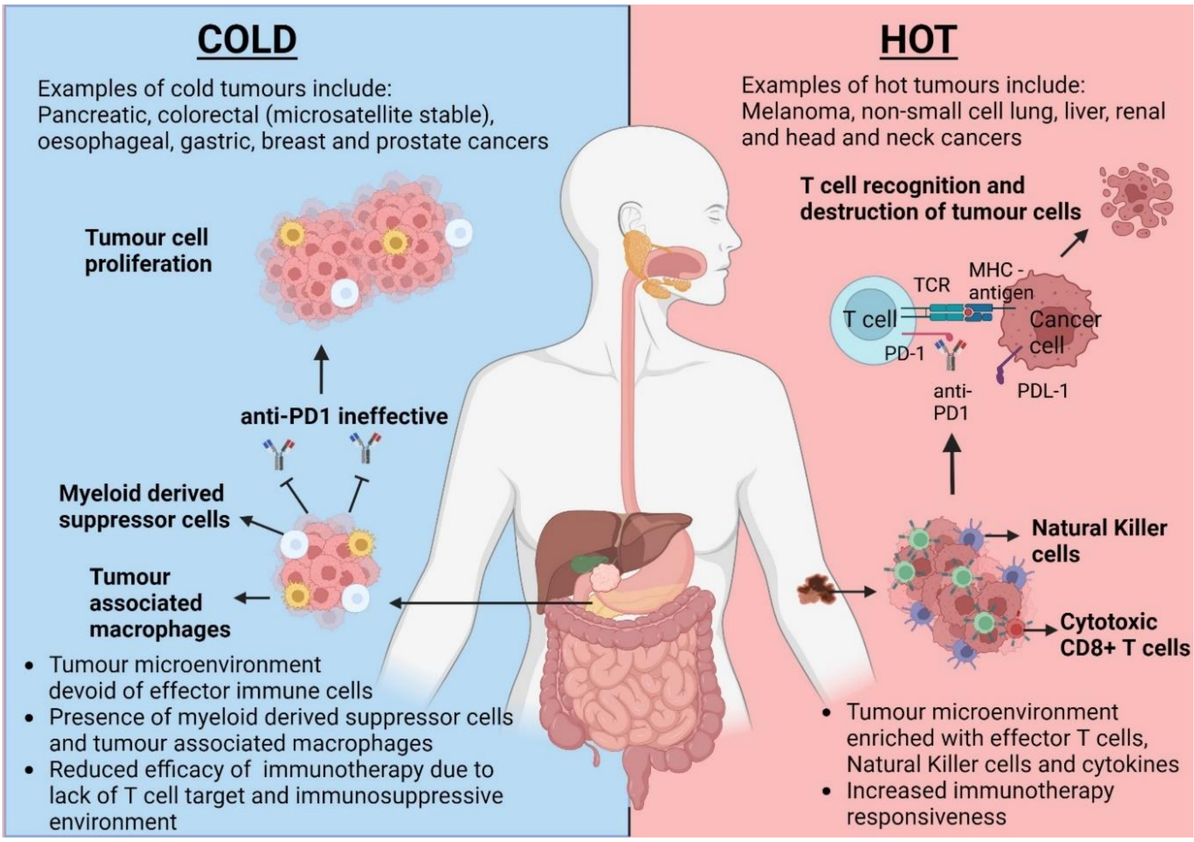


Figure 1.1: The opposing responses to immunotherapy dependant on the tumour microenvironment (TME) climate

This figure highlights the differences in TMEs between a classically hot tumour such as melanoma and a classically cold tumour such as pancreatic cancer. Intratumoral T cells in hot tumours provide a target for immunotherapies, such as anti-PD1, to allow inhibition of PD-1/PDL-1 interaction and allow subsequent cancer cell lysis. Due to the inherent lack of T cells within cold tumours, tumour cell proliferation remains uninhibited. *TCR – T cell Receptor, MHC – Major Histocompatibility Complex, PD-1 – Programmed Cell Death Protein 1, PDL-1 – Programmed Cell Death Ligand 1*

Kurz et al. demonstrated exercise induced benefits within different PDA mouse models showing tumour mass was decreased by 20-30% in exercised mice with their methodology accounting for potential confounding factors of reduced body and muscle mass (25). The reasoning behind this was explored using single cell RNA sequencing, demonstrating that exercise caused the shift towards mature lymphoid populations, such as cytotoxic CD8 T cells whilst reducing MDSCs within the pancreatic TME (25), thereby reducing the immune evading ability of the tumour. Exercise reduced CXCR2 expression, which is a myeloid cell receptor involved in signalling pathways that enhance MDSC populations (25). Ex vivo studies demonstrated higher Ki-67 levels, indicating proliferative activity, in CD8 T cells when cultured with MDSC isolated from tumours from exercised mice compared to controls indicating increased T cell activity and a reduction in the MDSC immunosuppressive effect (25). To the best of our knowledge, this currently is the only mouse study demonstrating the direct anti-cancer activity of exercise on PDA and its ability to turn this cold tumour hot. Given the poor prognosis, survival outcomes and high morbidity and mortality, this warrants further evaluation including in human studies.

A favourable immune environment has also been demonstrated in human studies with significantly raised CD3+ and CD8+ T cells and lowered Tumour Necrosis Factor alpha (TNFα) levels seen in the exercise cohort of oesophageal cancer patients in the study by Zylstra et al. (14). These studies have demonstrated the promising abilities of exercise to allow effector T cell infiltration and MDSC depletion within cold TMEs creating hot tumours.

1.5: Exercise and myokines

Myokines, including IL-6, IL-7 and I-15, released on muscle contraction play a role in immune system mediation along with many other physiological processes and therefore play a critical role in exerting the systemic effects of exercise (4,26). As discussed before, many components of the immune system can exhibit opposing mechanisms of action. IL-6 is involved in both pro-inflammatory and anti-inflammatory pathways, and it is the mechanism of release along with local and systemic conditions and duration of exposure which skew the direction of immune activity with exercise-induced short-term rises in IL-6 demonstrating more of an anti-inflammatory effect (4). One in vitro study using LoVo cell line (colorectal cancer cell line KRAS mutant TP53 wildtype) incubated with human serum taken pre and post exercise, showed a 4.2 % reduction in cell proliferation following exercise and demonstrated a 24.6% rise in serum IL-6 post exercise (17). The study demonstrated, using recombinant IL- 6 on LoVo cells, that increasing IL-6 led to a proportionate decrease in LoVo cell proliferation and γ-H2AX expression which relates to DNA damage (17). This study supports exercise-induced IL-6 as having potential anti-tumour properties although the complex dynamics and interactions of exercise-induced IL-6 in vivo would need to be examined and compared to the pro-inflammatory carcinogenic and immunosuppressive actions associated with long-term exposure to IL-6, which has been documented in murine models as enhancing CCR5 expression and subsequent promotion of MDSC activity (27).

IL-7 and IL-15 play an important role in modulating T cell levels and activity and are released with muscle contraction during exercise (4). IL-7 is involved in early signalling pathways stimulating naïve T cell proliferation and along with IL-15, promotes effective memory T cell concentrations after exposure to antigen (4). Kurz et al. (25) demonstrated the key anti-cancer role exercise-induced IL-15 plays by blocking IL-15 downstream signalling in PDA models in vivo and negating the beneficial effects. Therefore, the release of IL-7 and IL-15 during exercise may help shape the immune landscape and turn cold tumours hot.

There have been studies identifying different, novel myokines which exhibit anti-tumour properties, such as the secreted protein acidic and rich in cysteine (SPARC) myokine (28). SPARC levels rise instantaneously with acute exercise with a gradual decline over six hours following exercise cessation (28). Colon-26 cancer cell proliferation was suppressed when mouse recombinant SPARC was added (28), however the suppression plateaued at SPARC concentrations higher than 2 μg/ml and it is therefore difficult to conclude that raised SPARC levels above baseline in humans would have a similar effect.

Kim et al. demonstrated a significant rise in myokines oncostatin M (OSM) and SPARC in patients with metastatic castrate resistant prostate cancer (mCRPC) who underwent a 6 month supervised exercise regimen combining resistance training with aerobic exercise (29). They showed reduced cell growth in vitro when DU145 prostate cancer cells were cultured with their myokine enriched serum compared to the control arm (29). However limitations include small sample size and hence an inadequately powered study and the lack of specificity correlating the rise in myokines and their subsequent signalling pathways to tumour inhibition, highlighting the need for further review of the potential cytotoxic mechanisms in prostate cancer with exercise induced myokines (29).

Irisin is another myokine which exhibits significant metabolic effects leading to increased energy expenditure with an immediate increase in circulatory levels demonstrated following both aerobic exercise and resistance training (30,31). Gannon et al. showed in an aggressive epithelial breast cancer cell line, a significantly reduced cell number and viability and increased apoptotic signalling with enhanced caspase-3/7 activity with human recombinant non-modified irisin (31). Liu et al. demonstrated irisin's inhibitory effects on cancer cell growth in pancreatic cancer cell lines (32). This provides convincing in vitro evidence behind irisin's anti-cancer potential although in vivo studies would further strengthen the correlation between exercise-induced irisin and tumour inhibition. In vivo studies would also allow exploration of how exercise-induced myokines modulate cold TME. Figure 1.2 summarises the effect exercise has on the proliferation and circulatory release of immune cells and myokines leading to eventual cancer cell death.

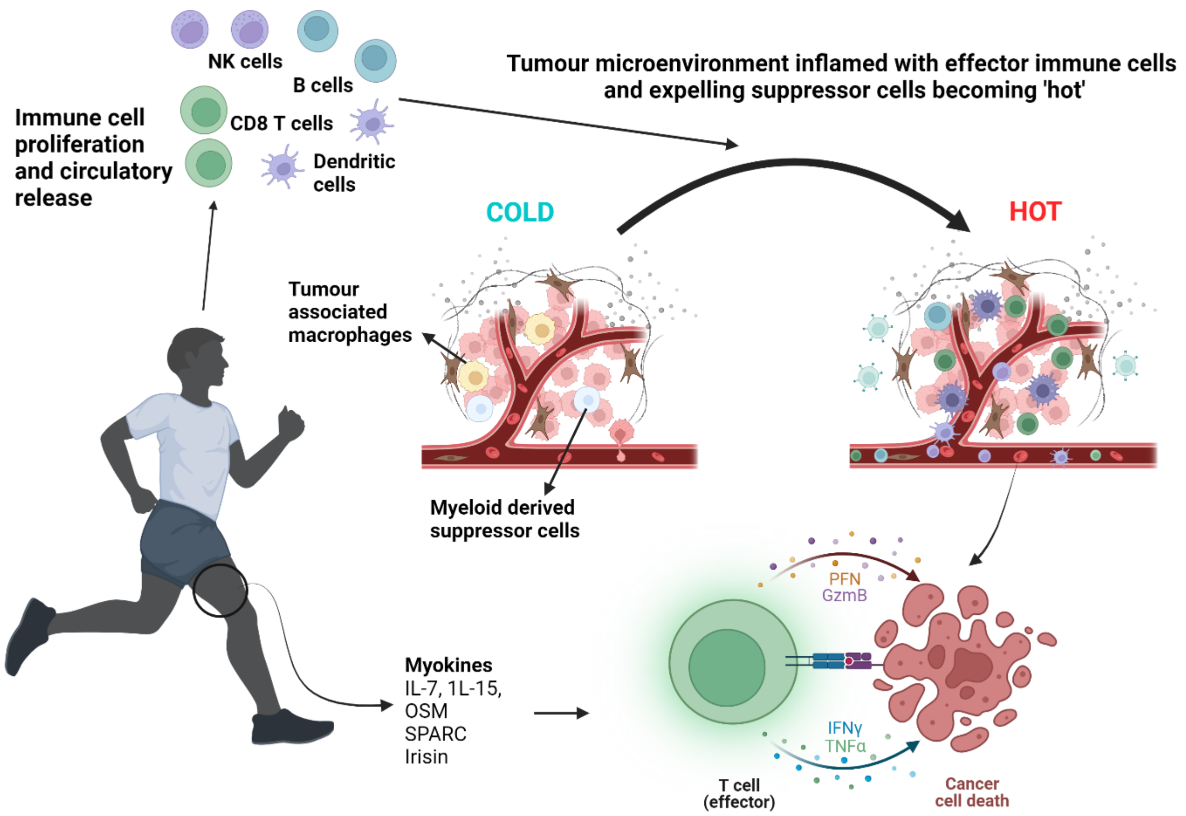


Figure 1.2: Exercise induced myokine and immune cell proliferation and release leading to T cell activation and cancer cell death

Aerobic exercise and resistance training mobilise myokines such as IL-7. IL-15, OSM, SPARC and Irisin alongside inducing a circulatory leucocytosis, including NK cells and T cells. The TME becomes 'inflamed' with effector immune cells. Effector T cell function is enhanced resulting in tumour lysis. *NK cells – Natural Killer cells, OSM – Oncostatin M, SPARC – Secreted Protein Acidic and Rich in Cysteine, PFN – Perforin, GzmB – Granzyme B, IFNγ – Interferon gamma, TNFα – Tumour Necrosis Factor alpha*

1.6: Exercise and the tumour vasculature

There is conflicting evidence with regards to vascular endothelial growth factor- α (VEGF-α) expression and exercise with both an up-regulation and down-regulation reported in the literature (33). VEGF- α is associated with angiogenesis and has been shown to be significantly raised in the immunotherapy groups with the combined exercise and nivolumab model in Martin-Ruiz's study having the highest VEGF- α tumoural levels as well as the highest necrotic index (33). One theory is that exercise leads to enhanced VEGF-α expression and so optimising intratumoural drug delivery (33).

C57BL/6 mice orthotopically implanted with transgenic adenocarcinoma of mouse prostate (TRAMP) C-1 cells undertook aerobic exercise using a voluntary wheel running method in a study exploring the association between exercise and tumour hypoxia and vessel structure (34). Within the TME, hypoxia fuels a signalling pathway resulting in raised hypoxia-inducible factor-1 (HIF-1) (34). Due to the dysregulated and exponential proliferation of cancer cells, tumour angiogenesis results in an inefficient and flawed vessel structure perpetuating an oxygen-depleted environment and tumour expansion (35).

However, this study noted a positive correlation between exercise and HIF-1α leading to enhanced VEGF expression within the TME along with ANGPT2, a gene marker of angiogenesis (34). They demonstrated using dynamic magnetic resonance (MR) scans, a significant increase in blood flow through tumours in exercised mice indicating vasculature normalisation (34,35). The authors hypothesise that exercise training may be linked to an alteration in the mechanism of action of HIF-1 combined with cytokine release to support tumour blood flow, illustrated in Figure 1.3 (34). This suggests tumour vasculature normalisation occurs secondary to the complex, synergistic interactions between exercise and the TME rather than a single pathway.

Bedoya et al. demonstrated that a home-based combined aerobic exercise and resistance training regimen prior to surgery for PDA exhibited a favourable change in the tumour vasculature with increased microvessel density and patency (15). Although exercise was performed concurrently with either chemotherapy, combination of chemotherapy and radiotherapy or during the recovery period between pre-operative treatment and surgery, the effect of exercise on treatment responses and clinical outcomes was not explored, subsequently missing a potential translational endpoint (15,36).

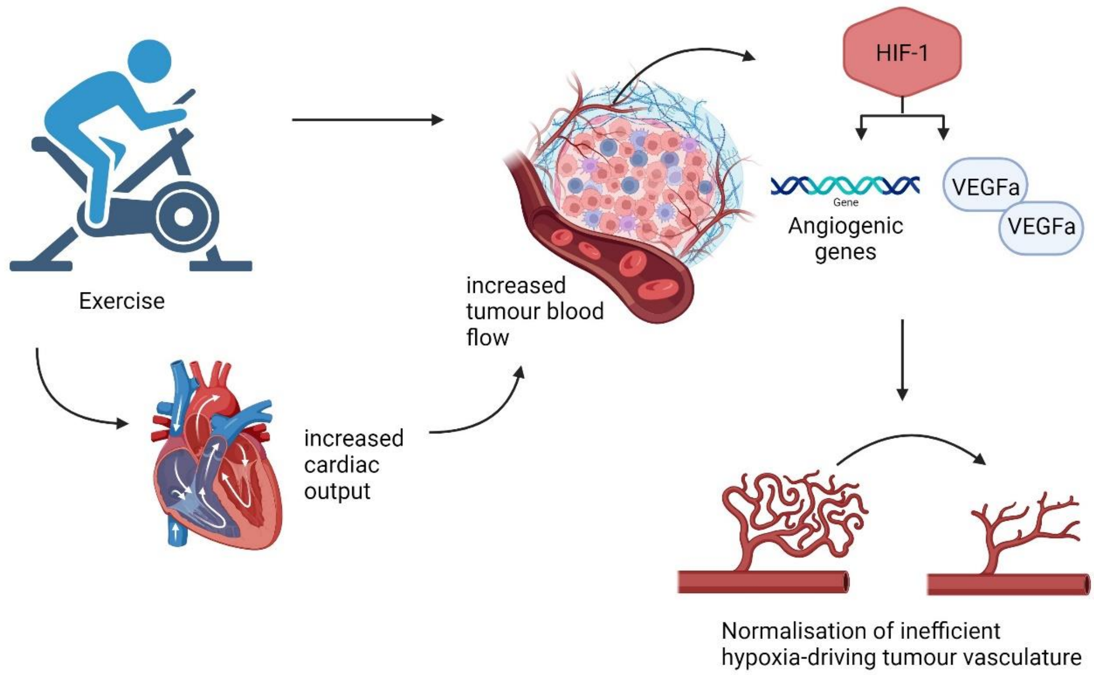


Figure 1.3: Exercise induced normalisation of tumour vasculature

An outline of some pathways that aerobic exercise may utilise to normalise tumour vessels, increase tumour blood flow and reduce hypoxia.

Exercise leads to increased cardiac output and a redistribution of blood flow away from constricted vessels within the splanchnic organs allowing supply to the contracting skeletal muscles (35). Betof et al. demonstrated, using the 4T1 breast cancer murine model, a significant reduction in EF5 (marker of hypoxia) within the tumours of exercised mice compared to sedentary mice (37). One theory behind the proposed benefit of exercise on tumour hypoxia includes the inherent nature of tumour vasculature to ignore vasoconstrictive instructions and consequently take advantage of the raised cardiac output and redirected blood flow (35).

Gomes-Santos et al. identified a simultaneous reduction in tumour hypoxia and an increased CD8+ T cell intratumoural influx with exercise training in mice implanted with breast cancer (38). Furthermore, Hatfield et al. were able to show that increasing the proportion of inspired oxygen in mice reduced tumour hypoxia and allowed a comparable increase in breast tumour infiltrating CD8+ T cells (39). These studies suggest that reduced tumour hypoxia could be a contributing factor to this CD8+ T cell tumour infiltration, providing a favourable hot TME for their stimulation and enhanced function (35). By modulating PD-L1 expression and CD8+ T cell infiltration, exercise-induced tumour oxygenation could potentially improve the efficacy of ICIs working within the PD-1/PD-L1 pathway (35).

1.7: Exercise and immunotherapy

Pre-clinical studies suggest exercise can modulate systemic and local tumour immunity by complex pathways upregulating immune components with anti-tumour activity, turning cold tumours hot and therefore, could play an adjunctive role to immunotherapy in patients with cancer.

Immunotherapy has revolutionised treatments across many different cancers and has shown to be particularly effective in hot tumours (5). ICIs, including anti- Programmed Cell Death Protein 1 (anti-PD1) and anti- cytotoxic T lymphocyte antigen 4 (anti-CTLA-4), are commonly used as standard of care in both curative and palliative settings. However, ICIs are not effective across all cancers, particularly cold tumours, highlighting the need for further research to reprogramme tumours to become more responsive to ICIs.

Martin-Ruiz et al. reviewed the effect of exercise (following inoculation with cancer cells) and nivolumab (anti-PD1) in NOD-SCID γ mice transplanted with human poorly-differentiated squamous non-small cell lung cancer (NSCLC), a hot tumour (33). A significantly raised necrotic index was observed in exercised mice with nivolumab compared with the non-exercise and isotype control group with the latter showing the greatest proliferating cell rate with Ki67 immunostaining (33). The necrotic index was not significantly different between the exercised and non-exercised groups receiving nivolumab and this may be due to the limited sample size (33). This study included combined aerobic and resistance training within the intervention; however we would be unable to draw any conclusions with regards to anti-cancer activity associated with resistance training alone. Proposed mechanisms of the anti-proliferative effects include promoting AMP-activated protein kinase (AMPK) signalling with subsequent suppression of the mammalian target of rapamycin (mTOR) oncogenic pathway (33,40). A statistically significant reduction in key components of mTOR activation was observed in a study where human exercised serum was applied to A549 NSCLC cells (33,40). Although no difference was seen in tumour growth or volume in mice receiving nivolumab irrespective of exercise, a reduced tumour growth rate and volume was found in the exercised mice in comparison to non-exercised mice, both groups having received isotype control (33). The immune deficient NOD-SCID γ mice would be unlikely to mount a sufficient response with anti-PD1 due to the inherent absence of T cells, providing difficulty in successfully evaluating the effect of exercise on cancer immunotherapy responses (41).

Buss et al. (41) examined EO771 'cold' breast tumour and B16-F10 'hot' melanoma mouse models and the interactions between post-implant exercise and anti-PD1 on local and systemic immunity, TME and growth. They observed a negative effect of exercise in these aggressive tumour models with a skew towards an immunosuppressive TME and exercise reduced CD8 T cell infiltration in the EO771 model and no effect on tumour growth seen when combined with anti-PD1 (41). Anti-PD1 (rat antibody designed to target mouse PD1) alone exerted no tumour inhibitory effects and TME modulation also occurred with the isotype control (41) and therefore brings in to question the validity of these results due to potential unaccounted for variables, including the validity of the anti-PD1 used. In clinical practice, pseudo-progression describes the inflammatory process with ICIs causing a paradoxical increase in assumed tumour volume, masking tumour response and may also provide a barrier to detecting the true anti-cancer effect of ICIs in murine models.
Wennerberg et al. demonstrated significant tumour growth inhibition with exercise during anti-PD-1 and radiotherapy treatment in a breast cancer murine model compared to sedentary mice receiving the same regimen (4,42). Similar to previous studies, exercise induced a local and systemic preference for anti-tumour immune cells and suppression of MDSC promoting the conversion of the breast cancer cold TME to a hot TME (4). Further murine breast cancer models have also shown that utilising exercise alongside ICIs enhanced tumour suppression (38). In clinical practice, we utilise the

combination of ICIs and chemotherapy for advanced triple negative breast cancer, however ICIs have shown little benefit outside of this sub-group, although multiple other treatments are available. Further studies into the role of ICI and exercise in breast cancer including hormone-positive and HER2-positive subgroups are required to support the clinical utility of exercise across all breast cancer sub-types.

Kurz et al. demonstrated that exercise optimised local immunity when combined with anti-PD1 by raising CD3 T cell and cytotoxic CD8+ T cell infiltrations and subsequently led to significant tumour size reduction compared to anti PD-1 alone in their PDA murine model (orthotopic transfer of KPC cells to wild type C57BL/6 mice) (25). The results are particularly promising due to the limited effective treatment for pancreatic cancer and the lack of responsiveness of cold tumours to immunotherapy, providing evidence that exercise could possibly expand the spectrum of tumours that could benefit from ICIs.

Table 1.1a: Pre-clinical studies with cold tumours (Hapuarachi et al)

Study authors	Cancer type(s)	Cell line / animal model / human	Exercise intervention	Study findings
In vitro				
Orange et al. (17)	Colon	LoVo	Males with risk factors for colon cancer (n=16). Moderately intense cycling (5-minute sessions performed 6 times with 2.5-minute breaks)	Serum of exercised individuals significantly decreased LoVo cell proliferation and γ-H2AX expression compared to serum collected prior to exercise. Exercise led to increased IL-6 levels.
Aoi et al. (28)	Colon	C2C12 myocytes and colon-26	Healthy males 30 minutes uninterrupted cycling at 70% maximum rate of oxygen consumption (VO2 max) Group 1: (n=10) One session Group 2: (n=9). Three weekly sessions for four weeks	Exercise increased SPARC levels in mice and humans following a single exercise session. SPARC reduced colon-26 cell proliferation.

Kim et al. (29)	Metastatic castrate-resistant prostate cancer (mCRPC)	DU145	mCRPC patients. Exercise group (n=13) 6 months of combination aerobic exercise (high-intensity, interval training) and resistance training. Control group (n=12) self-directed exercise	Supervised exercise training increased myokines oncostatin M (OSM) and SPARC compared to control. DU145 proliferation reduced with 6-month exercise-conditioned serum 12 to 61 hours after incubation but not at 72 hours.
Study authors	**Cancer type(s)**	**Cell line / animal model / human**	**Exercise intervention**	**Study findings**
Mouse studies				
Kurz et al. (25)	Pancreatic Ductal adenocarcinoma (PDA)	KPC cells in C57BL/6 mice	Running on a treadmill. 30 minutes, 5 times a week (mild intensity 15cm/s)	Exercise decreased tumour mass by 20-30%. Exercise increased intra-tumoural CD8+ T cells and reduced MDSCs with upregulation of 1L-15 signalling pathway.
Buss et al. (41)	Breast	Female C57BL/6 with orthotopic EO771 cells	Wheel running	Exercise did not affect NK cell levels. Exercise reduced CD8+ T cell proportions. Exercise combined with anti-PD1 had no significant effect on immune populations.
Wennerberg et al. (42)	Breast	4T1 in BALB/c mice	30 minutes 5 times a week. Running on a treadmill at 18m/min compared to control.	Exercise reduced tumour volume and MDSCs in the spleen. Exercise provided additional benefit in tumour inhibition when combined with radiotherapy and anti-PD1 compared

				to the latter two without exercise, as well as increased intratumoural NK cell activity.

Table 1.1b: Clinical studies with cold tumours

Study authors	Cancer type(s)	Cell line / animal model / human	Exercise intervention	Study findings
Human studies				
Zylstra et al. (14)	Oesophageal cancer	Oesophageal cancer patients for possible curative surgery	Combined moderately intense aerobic exercise/ weight training (n=21) compared to controls (n=19)	Enhanced pathological response and increased CD3 and CD8 T cells following pre-operative chemotherapy seen in the exercise group compared to control group.
Bedoya et al. (15)	PDA	Pancreatic cancer patients for possible curative surgery	70 patients undergoing neoadjuvant chemotherapy or combination chemotherapy /radiotherapy prior to surgery. Combination moderate level unsupervised aerobic exercise (1 hour per week of walking) and weight training (1 hour per week).	33/70 patients had their cancer surgery, although only 23 patients' pathology specimens were able to be included. Compared to non-exercised controls, exercise increased microvessel density and patency in the tumours. Exercise did not influence the amount of tumour regression.

Table 1.1c: Clinical studies with hot tumours

Study authors	Cancer type(s)	Cell line / animal model / human	Exercise intervention	Study findings
Mouse studies				

Pedersen et al. (13)	Melanoma, liver and lung	B16F10 in female mice, Diethylnitrosamine (DEN) in Naval Medical Research Institute NMRI male mice, Lewis Lung Carcinoma (LLC) in female mice	Wheel running. Average daily running distance per mouse: 4.1km (female) 6.8km (male)	All models: Tumour size, growth and metastatic disease reduced with exercise. B16 and LLC – increased pro- and anti-inflammatory immune components including IL-6 and NK cells.
Martin-Ruiz et al. (33)	Non-small cell lung cancer (NCSLC)	NOD-SCID gamma mice – patient derived xenograft model	40-60 minutes 5 times a week for 8 weeks. Combination treadmill running (performed at 40-80% maximal velocity) and resistance training.	Exercise led to reduced tumour growth and volume and in combination with nivolumab (anti-PD1), significantly increased the necrotic index comparative to double control, although necrotic index, cell proliferation and tumour growth were no different in the nivolumab groups irrespective of exercise.
Buss et al. (41)	Melanoma	Female C57BL/6 with subcutaneous B16F10 cells	Wheel running	Exercise did not affect NK cell levels. Exercise reduced CD8+ T cell proportions. Exercise combined with anti-PD1 had no significant effect on immune populations.

1.8: Discussion - Incorporating exercise into clinical practice

1.8.1: Exercise is feasible in the advanced cancer population

This review has discussed the different mechanisms by which exercise training can reprogramme the cold TME with effector T cell infiltration, MDSC depletion, anti-tumour myokine secretion and tumour vasculature normalisation creating a hot TME.

Mouse models of both cold and hot tumours have showed additional benefits of combing immunotherapy with exercise training. Further exploration of this potentially advantageous combination is required with human studies. There is a clear unmet need in patients with advanced cancer to improve overall response to immunotherapy and survival outcomes. Within the advanced cancer population, reduced functional reserve, exercise tolerance and respiratory function may provide significant obstacles to implementing exercise training into their cancer management.

Mikkelsen et al. showed that strength training in combination with a home-based walking programme over 12 weeks was feasible in an elderly population affected by either advanced pancreatic cancer, biliary tract cancers or non-small cell lung cancer (43). They demonstrated a 69% adherence rate and 94% completion rate of attended sessions occurring alongside first line palliative oncological treatment (43). The acceptability of this exercise intervention by an older age population and the inclusion of hard-to-treat advanced cold tumours is promising for the future incorporation of exercise into clinical practice in these populations.

A combination of cycling and resistance training was incorporated into a rehabilitation programme for patients with advanced lung cancers including mesothelioma (44). Only one third of eligible patients managed to be recruited with a 56.6% completion rate of the 8-week programme (44). This highlights the need to review reasons behind reduced participant acceptability of exercise training, cater interventions, and acknowledge limitations within the advanced cancer population.

1.8.2: Moderate intensity aerobic exercise and resistance training promote anti-cancer activity

Importantly, to incorporate exercise as an evidence-based standard intervention in cancer management, the FITT principles allow prescription of a measured dose of exercise, although there will still undoubtedly be challenges in determining the exact 'dose' of exercise required to exhibit treatment effect. Tables 1a and b outline pre-clinical and clinical studies researching the relationship between exercise and cancer in cold and hot tumours. The *in vitro* studies required pre- and post- exercise conditioned serum of healthy participants or patients with cancer depending on the study (17,23,28,29). The exercise regimes utilised varied from moderate –intensity to high-intensity interval training and largely centred on cycling. The window of opportunity studies (14,15) reviewing exercise as a prehabilitation measure in oesophageal and pancreatic cancer patients, respectively, used a combination of aerobic exercise and resistance training at moderate intensity, with Bedoya et al. incorporating a walking activity (15) and Zylstra et al. not specifying the type of aerobic exercise (14). All these studies were able to demonstrate a degree of intended benefit, however no direct comparisons from these studies between moderate and high intensity exercise can safely be drawn with regards to optimum anti-cancer activity. Further clarification with regards to a standardised effective dose is required to allow implementation of exercise as a prescribed intervention in clinical practice.

Animal studies in non-cancer models have shown that exercise training at moderate intensity, with a maximal oxygen uptake of around 70%, was able to promote cytotoxic immune function and support a Th1 cytokine profile, playing a protective role against infection (45). As immunity against infection overlaps with anti-tumour immunity, this further supports the use of moderate intensity exercise in cancer care.

Conversely, the opposite may be seen with chronic exercise at high intensity, for example in those at professional standards of exercise (45). After showing an initial increase within 30 minutes of high intensity endurance exercise, CD8+ T cells dropped along with Th1 cytokines IL2 and IFNγ (46). Previously, a 3-hour post exercise lymphopaenia had been addressed as potential redistribution from blood to tissue, however, a higher predisposition to developing infection has been noted (24,46).

Incorporating moderate intensity aerobic exercise for patients with cancer undergoing immunotherapy is likely to be more feasible and effective in promoting a favourable immune response. However, the translatability of these animal studies to patients with cancer should be considered given the differences in which an exercise intervention can be performed between humans and mice. Currently, there is limited evidence of the effectiveness of different exercise intensities within the advanced cancer population and is an area that warrants further exploration.

1.8.3: Future research

The utility of exercise as an effective intervention and an enhancer of immunotherapy responsiveness is being explored in ongoing human studies. ERICA is a prospective clinical trial looking into the practicalities and acceptability of undertaking exercise immediately prior to infusion of combination immunotherapy and chemotherapy in a French cohort of patients with metastatic NSCLC (47). Exploring the association between high intensity aerobic exercise and NK cell release in NSCLC, HI AIM is a randomised controlled trial that hypothesises augmented immunotherapy responses induced by exercise as a consequence of intratumoural and systemic immune cell influx (48). Importantly, these studies are investigating the clinical application of exercise in metastatic cancer populations, an area in oncology with vast room for improvement. NSCLC is typically a hot tumour and although there is scope for improving immunotherapy responses, to enhance the breadth of cancers that respond to immunotherapy, further research is required into cold tumours where immunotherapy is not effective.

Due to lack of efficacy, immunotherapy alone cannot be used as a comparator to an exercise – immunotherapy combination in cold tumours. Therefore, a clinical study assessing the latter combination in malignancies such as advanced pancreatic cancer, could be assessed as a maintenance strategy in patients that have completed first line palliative systemic anti-cancer treatment and have either responded or have stable disease. If the patient remains well and deemed appropriate by the clinical team, they normally will undergo surveillance with regular Computed Tomography (CT) scans to assess for disease progression. Figure 4. illustrates a potential multi-arm study design to explore exercise as an adjunct to immunotherapy in advanced pancreatic cancer in the maintenance setting following first line palliative treatment.

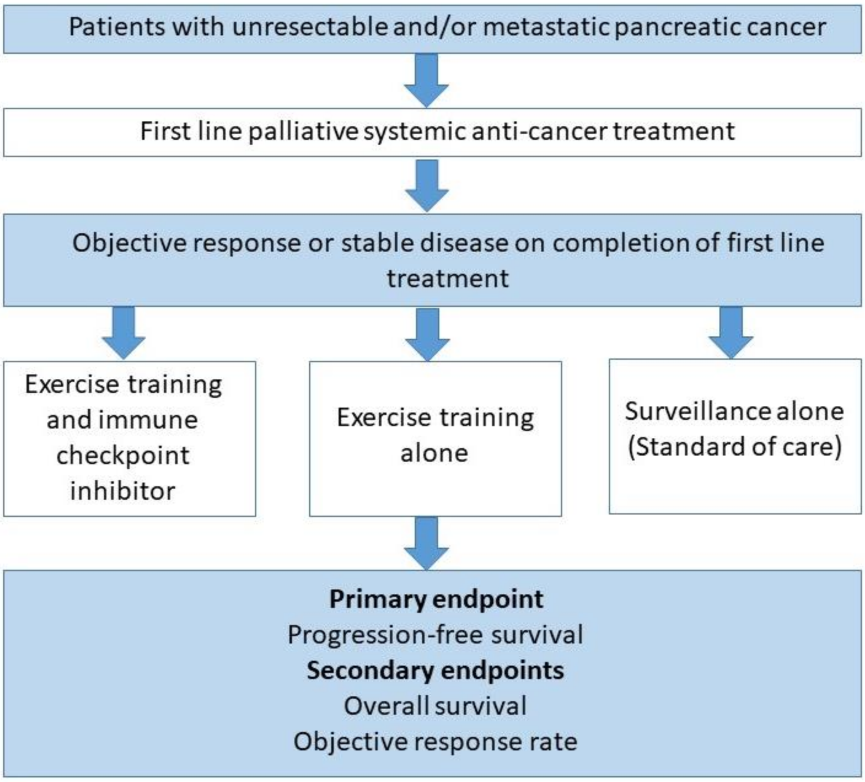


Figure 1.4: A multi-arm study design to explore the effectiveness of combing exercise training with immunotherapy as a maintenance strategy in previously treated advanced pancreatic cancer patients

A proposed randomised controlled trial design involving patients who have had an objective response or stable disease on completion of first line palliative systemic treatment for advanced pancreatic cancer. Patients would be randomised to either a combination of exercise training alongside a single agent immune checkpoint inhibitor or exercise training with standard of care or standard of care alone. All participants in each group will have regular Computed Tomography (CT) imaging every 3 months as standard of care. The primary endpoint would be progression-free survival (PFS), with overall survival (OS) and objective response rate (ORR) as secondary endpoints.

1.9: Conclusion

There is an increasing volume of evidence supporting various exercise-induced direct cytotoxic mechanisms in different cancer settings along with a crucial role exercise may play in local tumour and systemic immunity via anti-tumour immune cell infiltrations, favourable cytokine and myokine profiles and tumour vasculature normalisation (11,22,27).

Immunotherapy has paved the way for improving survival outcomes for many patients with cancer although immunotherapy-resistance and severe immune-mediated toxicities limit its universal utility. A significant barrier to immunotherapy effectiveness lies within the rigid, immunosuppressive and hostile TMEs of cold tumours such as pancreatic cancer (5,25). Evidence suggests that exercise training, including aerobic exercise and resistance training, may have a role in shifting tumours towards the hot end of the cold-hot TME spectrum. Exercise training could potentially be a promising, safe and cost-effective measure to overcome some of the obstacles to optimising immunotherapy responsiveness.

Within this PhD project, the effects of exercise on systemic immunity in healthy volunteers and on pancreatic cancer and mesothelioma in vitro and within in vivo models will be explored. The overall project hypothesis is that exercise combined with immunotherapy will enhance ‘anti-cancer’ immunity, improving treatment response in pancreatic cancer and mesothelioma.

Project aims:

1) To determine the effects of exercise on systemic immunity in healthy volunteers and exercise-conditioned serum on pancreatic cancer and mesothelioma cells in vitro (Chapter 3).
2) To examine the effect of exercise combined with an ICI on inducing tumour necrosis, reducing metastases and transforming TMEs towards a ‘hotter’ immune climate in pancreatic cancer and mesothelioma in mouse models (Chapter 4).
3) To design and set up a clinical feasibility study aiming to define a safe, feasible and effective dose of exercise in patients with advanced pancreatic cancer and mesothelioma, alongside anti-cancer treatment, that elicits the desired immune response (Chapter 5).

Chapter 2

Materials and Methods

2.0 Materials

Table 2.0.1: Cell lines

Cell line	Cancer type	Medium	Origin
MSTO-211H	Human biphasic mesothelioma	Roswell Park Memorial Institute (RPMI) 1640, 10% foetal bovine serum (FBS), 1% penicillin-streptomycin (P/S), 1% fungizone (F).	Obtained from collaborators, Dr Sarah Haywood-Small and Mr Sam Bonsall, at Sheffield Hallam University. Cells are of biphasic mesothelioma origin collected from the pleural fluid of a 62 year old white male (49).
PANC-1	Human pancreatic epithelioid carcinoma	Dulbecco's Modified Eagle Medium (DMEM; GIBCO), including 10% FBS and 1% P/S, 1% F	Provided by collaborators, Dr Helen Matthews and Dr Victoria Hart, at the University of Sheffield. Cells are of pancreatic epithelioid carcinoma origin arising from the pancreatic duct of a 56 year old white male (50).
Panc02	Mouse PDA	Dulbecco's Modified Eagle Medium (DMEM; GIBCO), including 10% FBS and 1% P/S, 1% F	Gift from Dr Ameera Jalini, University of Sheffield. Primary PDA in C57BL/6 mice (51).

AB-1	Mouse	RPMI 1640 with 10% FBS and 1% P/S	Obtained from Professor Paola Allavena, Clinical and Research Institute Humanitas, Rozzano (Milano, Italy). Derived from malignant ascitic fluid from a crocidolite asbestos-induced mesothelioma in BALB/c mice (52).
Vero	African green monkey kidney	DMEM-F12 including 10% FBS and 1% P/S, 1% F	Originated from African green monkey kidney in Chiba, Japan (53).

Table 2.0.2: Mouse strains

Mouse strain	Gender	Supplier
BALB/c	Male and female	Charles River Laboratory
C57BL/6	Male and female	Charles River Laboratory

Table 2.0.3: Healthy volunteer's study equipment

Equipment	Company
Exercise bicycle	Sunny Health and Fitness, California, USA
Medical tape	Medisave, Dorset, UK
Purple top BD Vacutainer EDTA Tubes	Medisave, Dorset, UK
Red top BD Vacutainer Heparin Tubes BD Vacutainer Eclipse blood collection needles with pre-attached holder	Medisave, Dorset, UK
Swabs	Medisave, Dorset, UK
Tourniquets	Medisave, Dorset, UK

Table 2.0.4: List of antibodies

Antibodies	Clone	Fluorophores	Reacts with	Company	Catalogue number
Annexin V	na	FITC	Human	BioLegend	640906
Calreticulin	1G6A7	AF647	Human	Novus	NBP1-47518AF647
CD3	UCHT1	FITC	Human	BioLegend	300406
CD3	17A2	PerCP/CY5.5	Mouse	BioLegend	100218
CD3	17A2	AF488	Mouse	BioLegend	100210
CD4	RPA-T4	BV510	Human	BioLegend	300546
CD4	GK1.5	FITC	Mouse	BioLegend	100406
CD8	SK1	AF700	Human	BioLegend	344724
CD8	53-6.7	BV605	Mouse	BioLegend	100744
CD8	53-6.7	PE	Mouse	BioLegend	100708
CD11b	M1/70	PE/ Dazzle 594	Mouse	BioLegend	101256
CD14	63D3	BV750	Human	BioLegend	367136
CD20	2H7	APC	Human	BioLegend	302310
CD25	S20019D	PEFire700	Human	BioLegend	385220
CD25	A18246A	BV421	Mouse	BioLegend	113705
CD45	2D1	PE	Human	BioLegend	368510
CD45	30-F11	BV785	Mouse	BioLegend	103149
CD56	W22097A	BV421	Human	BioLegend	343760
CD69	FN50	BV650	Human	BioLegend	310934
HLA-DR	L243	PECy7	Human	BioLegend	307616
Ly6C	HK1.4	AF700	Mouse	BioLegend	128024
NK1.1	PK136	APC	Mouse	BioLegend	108710
PD-1	29F.1A12	APC	Mouse	BioLegend	135210

Table 2.0.5: List of reagents

Reagent	Company	Location
Agarose	Sigma-Aldrich	Suffolk, UK
Alamar Blue	ThermoFisher Scientific	Loughborough, UK

Annexin V Binding Buffer	ThermoFisher Scientific	Loughborough, UK
BD™ Cytometric Bead Array (CBA) Human Flex sets (IL-5, IL-6, IL-10, IFNγ and TNFα)t	BD Biosciences	Wokingham, UK
BD Horizon, Brilliant Stain Buffer	BD Biosciences	Wokingham, UK
BD Pharm Lyse (Lysing Buffer)	BD Biosciences	Wokingham, UK
CellEvent™ Caspase -3/7 Red Detection Reagent	ThermoFisher Scientific	Loughborough, UK
Crystal Violet (0.2 %)	Sigma-Aldrich	Suffolk, UK
Dimethyl sulfoxide	Sigma-Aldrich	Suffolk, UK
D-Luciferin potassium	Abcam	Cambridge, UK
Dulbecco's Modified Eagle Medium (DMEM)	Gibco	Loughborough, UK
Dulbecco's Modified Eagle Medium F-12 (DMEM/F-12)	Gibco	Loughborough, UK
Formaldehyde paraformaldehyde (4 %)	ThermoFisher Scientific	Loughborough, UK
Fungizone	Lonzo, BioWhittaker Ltd	Wokingham, UK
Isoflurane	Teva UK Ltd	Castleford, UK
Matrigel Matrix	Sigma-Aldrich	Suffolk, UK
Mitomycin C	Sigma-Aldrich	Suffolk, UK
OneComp eBeads	Thermo Fisher Scientific	Loughborough, UK
Penicillin / Streptomycin	Lonzo, BioWhittaker Ltd	Wokingham, UK
Phosphate buffered saline	Lonzo, BioWhittaker Ltd	Wokingham, UK
Roswell Park Memorial Institute (RPMI) medium	Gibco	Loughborough, UK
Trypan Blue (0.4 %)	Sigma-Aldrich	Suffolk, UK
Trypsin-EDTA	Lonzo, BioWhittaker Ltd	Wokingham, UK
Tween 20	Sigma-Aldrich	Suffolk, UK
Xylene	Sigma-Aldrich	Suffolk, UK
Zombie dye near infrared (NIR) dye	Biolegend	London, UK

Table 2.0.6: List of biological reagents

Biological reagent	Company	Location
HSV1716	Virttu	Glasgow, UK
HSV1716-GFP	Virttu	Glasgow, UK

Table 2.0.7: List of equipment and instruments

Equipment	Company	Location
AE2000 light microscope	Motic	Barcelona, Spain
Automated Haemocytometer	BIO-RAD	Watford, UK
Cytek Aurora Flow Cytometry System	Cytek Biosciences	Cambridgeshire, UK
Dual-chamber cell-counting slide	BIO-RAD	Watford, UK
Ensight Multimode Microplate reader	PerkinElmer	
Eppendorf Centrifuge 5702	Eppendorf	Stevenage, UK
Invitrogen™ EVOS™ M7000 Imaging System	ThermoFisher Scientific	Massachusetts, USA
Horizontal wired screen	University of Sheffield	Sheffield, UK
Incubator	SANYO	
LSM 980 Airyscan Confocal microscope	Zeiss	Cambridge, UK
LSRII Flow Cytometer	BD Biosciences	Wokingham, UK
MSE MISTRAL 2000 Centrifuge	MSE	Cholet, France
Mouse treadmill	Panlab	Barcelona, Spain

2.1: Cell culture

All cell lines used throughout this research project can be found in Table 2.0.1. Cells were incubated within a SANYO incubator set at 37ºC and 5% carbon dioxide (CO_2) in T75 flasks. Cells were reviewed daily and if deemed to be 70-80% confluent, cells were passaged. This process included discarding the old medium, washing with 5ml of phosphate buffered saline (PBS) and then incubating the adherent cells at 37 ºC with 3ml of 0.05% trypsin for 5 minutes. Using an AE2000 light microscope (Motic), detachment of the cells was confirmed. 5ml of fresh complete medium was then added and the cell suspension transferred to a 15ml falcon tube. Then using either the MSE MISTRAL 2000 Centrifuge or Eppendorf Centrifuge 5702, cell suspensions were spun at 1000 RPM for 5 minutes. The supernatant was discarded and cells were resuspended in 5ml of fresh complete medium.

For the MSTO-211H cells, 1:16 dilution, using further medium, was usually performed with transfer to a new T75 flask. For PANC-1 cells a 1:4-1:5 dilution was performed. For Panc02, AB-1 and Vero cells, a 1:10 dilution was performed. All cell lines were cultured up to 30 passages. All cell lines underwent regular mycoplasma testing.

2.2: Healthy volunteer study

2.2.1: Sample size

A sample size calculator from clincalc.com (Sample Size Calculator (clincalc.com)) was used to determine the number of participants required for this study. Similar to Orange et al.'s study on LoVo colorectal cancer cell viability with exercise-conditioned serum (17), assuming a 54% +/- 3% viability, 18 participants were required to demonstrate a 4% decrease in viability with exercise-conditioned serum with alpha level of 0.05 at 80% power.

2.2.2: Ethics

Ethical approval was achieved from the University of Sheffield in January 2023 following an application (reference number 050457, appendix A) detailing clear aims and objectives, methodology, recruitment processes, safety and risk assessments, data processing and storage as well as participant information sheets (appendix E) and consent forms (appendix H).

2.2.3: Aims and Objectives

1) To assess the effect of exercise-conditioned human serum on mesothelioma and pancreatic cancer cell lines on cell viability, migration and apoptosis.

2) To assess the effect of exercise on peripheral blood mononuclear cell (PBMC) subsets by flow cytometry.

3) To assess the effect of exercise on pro- and anti-inflammatory cytokines measured by cytokine bead arrays.

2.2.4: Recruitment

Healthy volunteers were recruited from the staff and student population at the University of Sheffield. Potential participants were emailed and provided with a participant information sheet along with contact details. If interested, participants had a further verbal discussion of the study and a written informed consent form was signed by the participant and myself, as lead researcher.

2.2.5: Inclusion criteria

Healthy volunteers over the age of 18 years old with no significant medical conditions and deemed not to have any risk associated with high intensity interval aerobic exercise on an exercise bicycle.

2.2.6: Exclusion criteria

Any medical conditions that would mean high intensity aerobic exercise would be pose a high risk. These included, but were not limited to, angina, recent heart attack or any other heart conditions, recent stroke, recent bone fracture, central nervous system disorders, poorly controlled asthma and uncontrolled diabetes mellitus.

2.2.7: Exercise activity

Participants underwent a single high intensity interval aerobic exercise session on an exercise bicycle. This involved 2 minutes of gentle cycling as a warm up, followed by 4 minutes of cycling at high intensity and then 2 minutes of gentle cycling or rest. The 4-minute-high intensity cycling was then repeated to a total of three times. Intensity was judged subjectively using the Borg scale ranging from 6-20 to assess

Rate of Perceived Exertion (RPE). The table below describes what each score indicates. Participants were aiming to reach a score of 16-20 to ensure they were performing at high intensity.

Table 2.1: Borg Scale to assess Rate of Perceived Exertion (RPE) (50)

Score	Degree of exertion
6	No exertion
9	Very light exertion (e.g. Walking short distances)
12-14	Moderate intensity (e.g. Jogging)
16-20	High intensity (e.g. Sprinting)

Blood tests were taken prior to and immediately after the exercise session. At each time interval, 4ml of blood was collected in an ethylenediaminetetraacetic acid (EDTA) containing blood tube and 10ml of blood was collected in a red-top blood tube containing no additives. EDTA blood tubes were placed on ice whilst red-top tubes were kept at room temperature allowing blood to coagulate.

2.3: Flow Cytometry

A flow cytometry panel was designed to include 10 leucocyte antibody markers (BioLegend) and a live/dead Zombie Near-Infrared (NIR) dye. The following fluorophores were chosen to avoid spectral overlap, demonstrated in Figure 2.1 to separate and quantify different leucocyte subtypes (shown in Table 2.2).

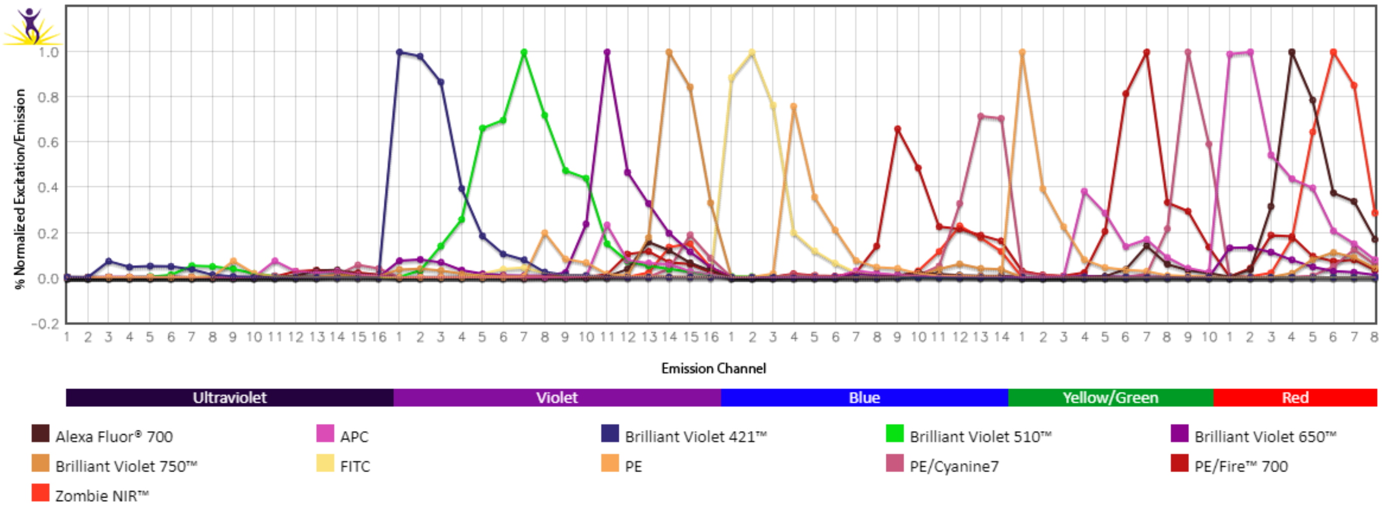


Figure 2.1: BioLegend's Aurora Spectra Analyser

Emission spectra of all fluorophores within the flow cytometry panel illustrated using BioLegend's Aurora Spectra Analyser.

Table 2.2: Flow cytometry panel containing 10 antibody-fluorophore conjugates

Antibodies	Description	Fluorophores
CD3	General T cell marker	FITC
CD4	T helper cells	BV510
CD8	Cytotoxic T cells	AF700
CD14	Monocytes	BV750
CD20	B cells	APC
CD45	General leucocyte marker	PE
CD56	Natural Killer (NK) cells	BV421
CD25	T cell activation marker	PEFire700
CD69	Early T cell activation marker	BV650
HLA-DR	T cell activation marker	PECy7

Optimisation steps including antibody titration and fluorescence minus one (FMO) were performed to identify ideal antibody concentrations and provide reference values.

2.3.1: Optimisation: Antibody titration

The recommended manufacturer suggestions (BioLegend) were to use 5µl of each antibody conjugate for every 100µl of whole blood. Whole blood was collected from a healthy volunteer into three EDTA blood tubes containing 4ml of blood each. 200µl of blood was added to 34 separate 15ml falcon tubes followed by 2.5µl of human BD Fc block (BD Biosciences) and left at room temperature for 10 minutes to prevent non-specific binding.

For each of the ten antibody-fluorophore conjugates and the Zombie NIR dye, three Eppendorf tubes were used. 200µl of Fluorescence-activated Cell Sorting (FACS) buffer (2% FBS, 98% PBS) was added to the first Eppendorf tube followed by either 10µl of the antibody or 4µl of the Zombie NIR dye. Serial dilutions were then performed twice with 100µl of the antibody or dye / FACS buffer solution pipetted into the next Eppendorf tube and diluted with a further 100µl of FACS buffer. Therefore, for each antibody-fluorophore conjugate, there were three Eppendorf tubes containing 5, 2.5 and 1.25µl per 100µl of FACS buffer respectively. Serial dilutions of Zombie NIR dye were performed in the same manner to produce three Eppendorf tubes with 2, 1 and 0.5µl per 100µl of FACS buffer respectively.

100µl of each antibody or Zombie dye dilution was added to one of the 34 separate 15ml falcon tubes containing 200µl of blood. The last falcon tube was used as a control and 100µl of FACS buffer with no antibodies was added (unstained). This was then incubated on ice in the dark for 20 minutes.

2ml of 1X Lysing Buffer (BD Pharm Lyse; 10x concentration reconstituted with 1 part 10X Pharm Lyse and 9 parts distilled water) was then added to each falcon tube and incubated at room temperature in the dark for a further 15 minutes. Each sample was then spun at 1000 RPM for 5 minutes; the supernatant was discarded and the pellet washed with 2ml of PBS before being spun again at 1000 RPM for 5 minutes. The washing process was repeated twice prior to addition of 350µl of 1% paraformaldehyde (PFA) (ThermoFisher Scientific; 4% PFA reconstituted with 1 part 4% PFA and 3 parts PBS). Samples were then incubated at room temperature for 30 minutes. Following this, each sample was washed once with 2ml of PBS in the same process explained above and finally suspended in 350µl of PBS and then kept in a 4ºC fridge and analysed the next day.

The optimum antibody concentrations for each antibody-fluorophore conjugate and Zombie NIR dye were determined using the Cytek ® Aurora Flow Cytometry System and are listed in the table below.

Table 2.3: Optimum antibody-fluorophore concentrations

Antibody-fluorophore conjugate	Volume required (per 100µl of blood)
CD3 - FITC	2.5µl
CD4 - BV510	2.5µl
CD8 – AF700	2.5µl
CD14 – BV750	2.5µl
CD20 - APC	2.5µl
CD45 – PE	2.5µl
CD56 – BV421	2.5µl
CD25 – PEFire700	2.5µl
CD69 – BV650	2.5µl
HLA-DR – PECy7	1.25µl
Zombie NIR dye	1µl

2.3.2: Optimisation: fluorescence minus one (FMO)

The final optimisation step was performing FMOs to allow the accurate gating and quantification of each leucocyte antibody in the study. 100µl of Brilliant stain buffer (BD Biosciences) was added to 13 Eppendorf tubes. One tube was labelled 'unstained' and no further reagents were added. The previously stated optimal concentration of each antibody / Zombie NIR dye was added to one of the Eppendorf tubes (labelled 'all antibodies'). All antibodies/ Zombie NIR dye except for one were then added to each of the remaining 11 Eppendorf tubes with each tube missing a different antibody and one tube containing all antibodies with no Zombie NIR dye. The same process described above was then performed with the 13 falcon tubes using 200µl of blood.

2.3.3: Flow cytometry preparation and serum separation for the healthy volunteer study

Coagulated blood from red-top tubes (pre and post exercise) were centrifuged at 2000 RPM for 10 minutes at 4ºC. Serum was separated using a Pasteur pipette and aliquoted into cryovials, frozen and stored in a -20ºC freezer for later use in cell culture and cytokine analysis.

200µl of blood taken before and after exercise for each participant was incubated with all 10 optimal antibody-fluorophore conjugate concentrations and Zombie NIR dye in the same process detailed above. Following fixing in 1% PFA, cells were washed with and resuspended in PBS, kept in a 4ºC fridge overnight and analysed the next day using the Cytek Aurora Flow Cytometer.

Changes in cell counts for each leucocyte population with exercise were analysed using GraphPad Prism 8.0.2.

2.4: Cell viability

Cell viability assays were performed by myself and Hannah Brown, Master of

Science (Research) student in Translational Oncology, University of Sheffield (March- August 2023), who I supervised on this project.

For tumour cell viability assays, mesothelioma (MSTO-211H) and pancreatic cancer cells (PANC-1) were incubated with serum from participants taken before and after exercise in 96 well plates. Cell viability was determined using alamarBlue® (BIO-RAD), whose active compound resazurin (blue and not fluorescent) is converted to resorufin (red and very fluorescent) in viable cells (54). Once cells reached around 70-80% confluence in their T75 flask, the medium was discarded, the adherent cell layer was washed with 5ml of PBS and then incubated with 3ml of 0.05% trypsin for 5 minutes. 5ml of fresh complete medium was then added and the cell suspension transferred to a 15ml falcon tube. Then the cell suspension was spun at 1000 RPM for 5 minutes. The supernatant was then discarded, and cells were resuspended in 5ml of fresh complete medium. 10µl of the new cell suspension was then taken and diluted with 90µl of PBS, making a 10% solution. 10µl of the 10% solution was added to 10µl of trypan blue and positioned on a dual-chamber cell-counting slide (BIO-

RAD) and using a haemocytometer, cells were counted. Once given a figure of cells per 1 ml, this was then multiplied by 10 to account for the previous dilution to 10%. A 96-well plate was then seeded (in columns 2-10) with 5000 cells (either MSTO-211H or PANC-1) in 100μl of their complete medium (10% FBS and 1% penicillin-streptomycin). Column 11 contained 100 μl of complete medium alone. The outer wells were avoided due to potential evaporation. The plate was then incubated at 37 ºC for 24 hours.

After 24 hours, cell adherence to the wells was confirmed using the AE2000 light microscope (Motic). The medium was discarded, and each well was washed with 100μl of PBS, which was then discarded. Each plate was incubated with the serum of one participant. The figure below outlines the strategy used to incubate the cells. Two plates were made for each participant.

Serial dilutions of serum with medium was performed and dilution ratios of 1:10, 1:50, 1:100, 1:500, 1:1000 and 1:5000 were used. In order to prepare the dilutions required for two plates, 900μl of cell specified medium (with 1% penicillin / streptomycin and 1% fungizone, without FBS) was added to 100μl of either pre- or post- exercise participant serum creating a 1:10 dilution of the serum. 80μl of 1:10 solution was taken and added to 720μl of medium (without serum) to create a 1:100 dilution and a further serial dilution with same volumes used to make a 1:1000 dilution. To make 1:50 dilution, 600μl of medium (without serum) was added to 150μl of 1:10 solution and then serial dilutions, as above, performed to create 1:500 and 1:5000 dilutions. This would then allow 100μl of each dilution to be added to a well, and to be incubated in triplicate on each plate, as shown in Figure 2.2. The same dilution process was used with complete medium.

One plate was incubated until 24 hours and the other was incubated for 48 hours before 10μl of alamarBlue® was added. After 4 hours of incubation with alamarBlue®, fluorescence was read using the Ensight Multimode Microplate Reader, with a higher fluorescence reading indicating a higher cell viability. Upon review of the well plates, additional 72-hour plates were performed to assess whether statistically significant changes in cell viability required a longer incubation to become evident. GraphPad Prism 8.0.2 was used to analyse differences in fluorescence of cells treated under the different conditions.

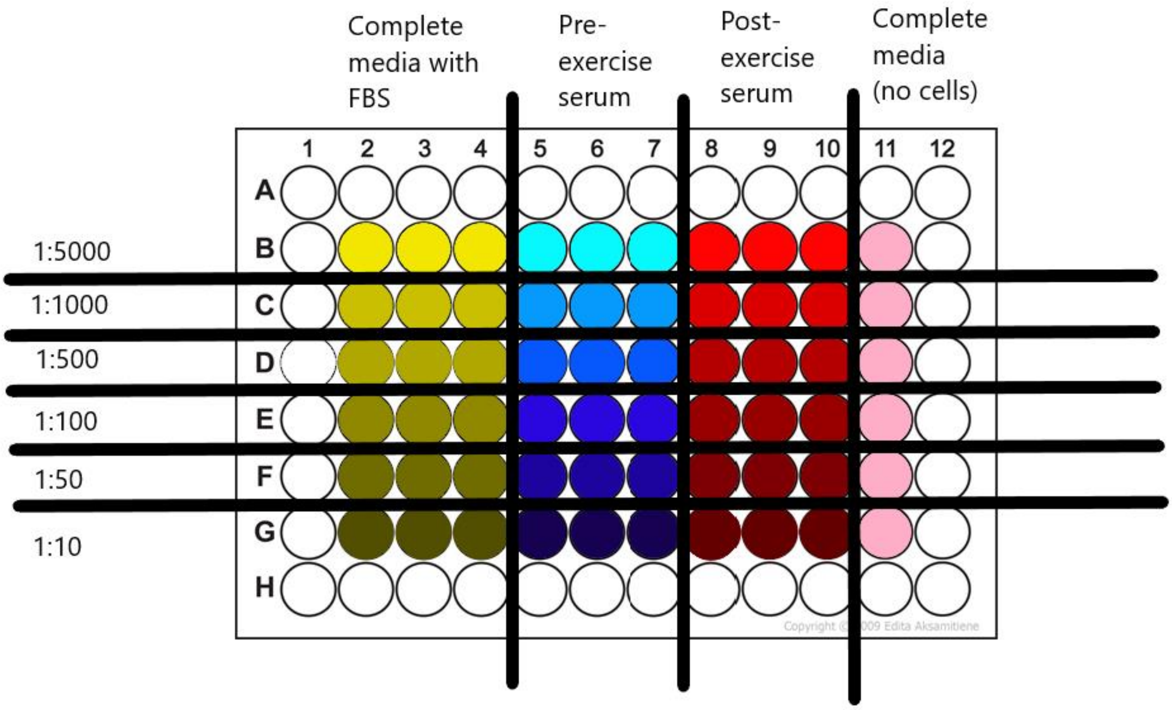


Figure 2.2: 96-well plate incubating strategy

96 well template from [Barrick Lab:: ProtocolsFluorescentProteinEvolutionaryStability](), demonstrating how cells were cultured in triplicate with different sera and at different dilutions.

2.5: Scratch assays

Scratch assays were performed by myself and Hannah Brown, Master of

Science (Research) student in Translational Oncology, University of Sheffield (March- August 2023), who I supervised on this project.

2.5.1: Optimisation

Optimisation steps were carried out to determine the number of PANC-1 and MSTO-211H cells required to seed one well in a 48 well plate to create a confluent layer in 24 hours. Initially, 1.5 $x10^4$, 3 x 10^4 and 5 x 10^4 PANC-1 and MSTO-211H cells were seeded in separate wells and confluence was not reached in 24 or 48 hours. A 10µl pipette tip was used to create a scratch on the bottom of the wells using the plate lid as a straight edge to scratch a line. However, due to the fragility and instability of the 10µl pipette tip, we were unable to scratch a clear line on the well plate.

Following this, 5 x 10^5 and 1x 10^6 PANC-1 and MSTO-211H cells were seeded. Outer wells were avoided. 100% confluency was achieved at 24 hours for all wells from both cell types.

After 24 hours, medium was discarded from each well and 6µl (12µg) of 2mg/ml mitomycin C was added to 6ml of serum free medium creating a 2µg/ml mitomycin C solution. Then 300µl of this was added to each well to prevent cell proliferation and the plate was incubated at 37 ºC for 90 minutes. This step was to ensure that any cell movement across the scratch was due to cell migration and not cell proliferation.

After 90 minutes, medium was removed and cells were observed under the AE2000 light microscope (Motic). The majority of MSTO-211H cells had lifted, indicating cell death following the mitomycin C with a smaller number of lifted cells seen with the PANC-1 cells.

2.5.2: Troubleshooting

Further steps included adding 4µl (8µg) of 2mg/ml mitomycin C to 6ml of serum-free medium creating a 1.3µg/ml mitomycin C solution. Cells were then incubated with 300µl of this solution at 37 ºC for 90 minutes. MSTO-211H cells continued to lift, whereas all PANC-1 cells remained adherent. 1µl (2µg) of 2mg/ml mitomycin C was added to 6ml of serum free medium (DMEM) creating a 0.3µg/ml mitomycin C solution to be used only with MSTO-211H cells. Using the same incubation process, the majority of cells were adherent after 90 minutes. On removal of the medium, a 200µl pipette tip was used to create the scratch with the edge of the tip cut using scissors. Following the scratch, MSTO-211H cells lifted.

Another method to prevent cell proliferation instead of mitomycin C was attempted using a starvation period. Following confluency of the MSTO-211H cells in the 48 well plate, medium was discarded and replaced with serum-free medium. On review after 90 minutes, the cells appeared to be confluent but following the scratch using a 200µl pipette tip, most cells lifted. Therefore, scratch assays were not performed with MSTO-211H cells.

In order to further assess the cell starvation method, PANC-1 cells were starved following the same protocol after reaching confluency at 24 hours. After 90 minutes, the cells had mostly lifted. Therefore, mitomycin C was used to inhibit cell proliferation at a concentration of 1.3 µg/ml for all scratch assays performed with PANC-1 cells.

Scratches within the wells seeded with PANC-1 cells were visualised and imaged using the EVOS™ FL Auto Imaging System (ThermoFisher Scientific). In order to image the same area within the well at different time points, routines were created for each plate. This involved selecting two beacons at opposing corners of the area, on each well, required to image. These beacons were meant to depict the same area when the routine was run at different time points, allowing direct comparison of the same area of scratch. However, on several occasions, the beacons moved between different time points, rendering images invalid for further analysis. We attempted to adjust the location of the beacons if noted to have moved, however this was unsuccessful. The EVOS microscope was then replaced with Invitrogen™ EVOS™ M7000 Imaging System (ThermoFisher Scientific). Further scratch assays were performed with the new EVOS microscope with no issues.

2.5.3: Analysis

Valid images were analysed using ImageJ 1.52g (National Institutes of Health) with the area of patent scratch in each image measured and documented. Percentage changes in scratch area were calculated at each time point and analysed using GraphPad Prism 8.0.2. Wilcoxon signed-rank tests were used to assess paired data for statistical significance (pre- and post-exercise conditioned serum).

2.6: Transwell migration assays

MSTO-211H cells were cultured with Vybrant DiD Cell-Labelling solution for 10 minutes in serum-free media. After washing in PBS, $1x10^5$ MSTO-211H cells in 100µl of complete RPMI medium were seeded on 3.0µm pore cell culture inserts and placed in a 24 well plate. After 10 minutes of incubation at 37°C, wells for filled with either 600µl of complete medium (control), or 540µl of serum-free medium with 60µl of pre- or post-exercise conditioned serum (n=8). Plates were incubated for 2 hours, inserts removed and cotton-tipped applicators used to remove non-migrated sides from apical membrane. Migrated cells on the basal membrane were fixed with 4% PFA. Fluorescent Images of basal membrane taken using Invitrogen™ EVOS™ M7000 Imaging System (ThermoFisher Scientific). This methodology is summarised in Figure 2.3. Images were analysed and migrated cells counted using ImageJ 1.52g (National Institutes of Health). Differences in cell migration were analysed using GraphPad Prism 8.0.2 and Kruskal-Wallis test.

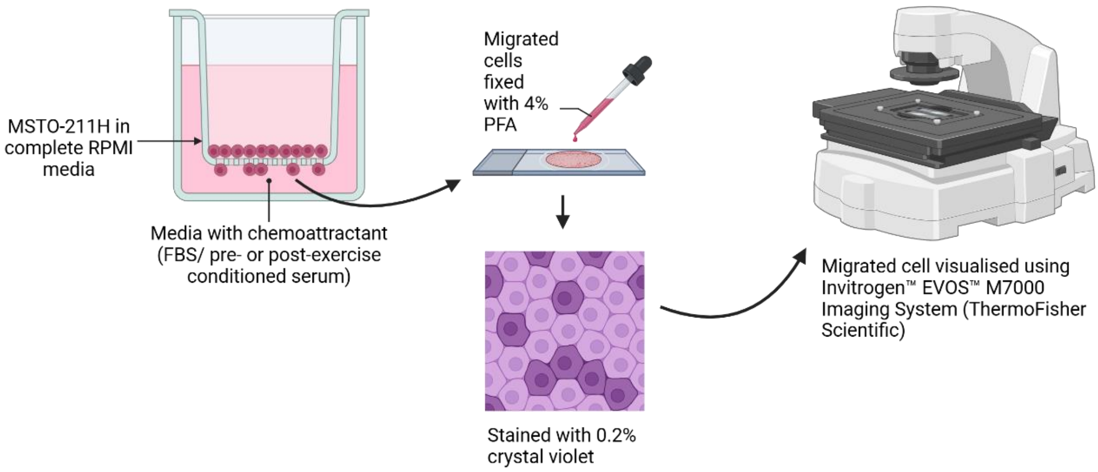


Figure 2.3: Transwell migration assay of MSTO-211H mesothelioma cells

$1x10^5$ MSTO-211H cells in 100µl of complete RPMI medium was seeded on 3.0µm pore cell culture inserts and placed in a 24 well plate. After 10 minutes of incubation at 37°C, wells for filled with (A) 600µl of complete medium (control), or 540µl of serum-free medium with 60µl of (B) pre- or (C) post-exercise conditioned serum (n=8). Plates were incubated for 2 hours, inserts removed, and cotton-tipped applicators used to remove non-migrated sides from apical membrane. Migrated cells on the basal membrane were fixed with 4% PFA. Cells were then stained with 0.2% crystal violet.

2.7: Cytokine bead arrays

Cytokine bead arrays were carried out on the pre- and post- exercise conditioned serum of five participants within the healthy volunteer study. We used a panel of antibodies detecting IL-5, IL-6, IL-10, IFNγ and TNFα. Senior Technician, Sue Gauntlett, performed this along with myself and our MSc student, Hannah Brown.

Initially, a standard containing 2500 picograms/ml (pg/ml) of each cytokine was added to one tube with 4ml of assay diluent and placed in the fridge for 30 minutes.

Serial dilutions of the standard were performed in the same method as described previously to produce 10 tubes with 25µl of the following concentrations of each cytokine (run in duplicate): 0pg/ml, 10pg/ml, 20pg/ml, 40pg/ml, 80pg/ml, 156pg/ml, 312pg/ml, 625pg/ml, 1250pg/ml and 2500pg/ml.

25µl of either pre- or post-exercise conditioned serum from each participant was added to 2 different tubes (20 tubes in total, 4 for each of the 5 participants with 2 containing pre-exercise conditioned serum and 2 containing post-exercise conditioned serum).

30µl of each of the 5 cytokine capture beads was added to a tube to produce 150ul of capture bead solution, which was diluted with 1350µl of capture bead diluent. 25µl of this solution, containing 0.5ul of each cytokine capture bead, was added to the 10 tubes containing the standard serial dilutions as well as the 20 tubes containing serum. Samples were then incubated at room temperature in the dark for 1 hour. 25µl of reaction agent was then added to each sample and incubated for a further 2 hours at room temperature.

Following this, samples were centrifuged at 200G for 5 minutes. The supernatant was discarded and the pellet was washed with 200µl of wash buffer. The LSRII Flow Cytometer was then used to analyse the cytokine bead arrays.

2.8: Apoptosis assays

2.8.1: Caspase 3/7 assays

Cell death marker assays were performed by myself and Hannah Brown, MSc student who I supervised on this project.

In order to assess apoptosis of PANC-1 and MSTO-211H cells when cultured with pre- and post-exercise conditioned serum, cells were treated with CellEvent™ Caspase -3/7 Red Detection Reagent. Wells in a 96-well plate were seeded with 5000 cells and grown in 100µl of complete medium for 24 hours until confluent. Complete medium was then discarded and then either further complete medium, pre-exercise conditioned serum (10% serum with medium) or post-exercise conditioned serum (10% serum with medium) was added and allowed to incubate for a further 24 hours. 100µl of PBS was added to the CellEvent™ dry powder to create a 100X concentrated solution. For each participant, 3µl of 100X solution was added to 27µl of either complete medium, medium with 10% pre-exercise conditioned serum or medium with 10% post-exercise conditioned serum to create 10X solutions. This allowed samples to be analysed in triplicate with 10µl of the 10X solution added to each well. Following 60 minutes of incubation at 37ºC, fluorescence was read using the Ensight Multimode Microplate reader. These assays were repeated using 6-7 different participants' pre- and post- exercise conditioned serum.

2.8.2: Annexin and Calreticulin assays

Further cell death marker assays were performed to further characterise the mechanisms of apoptosis induced by exercise-conditioned serum. 1 x 10^6 PANC-1 cells were seeded onto a 24-well plate for 24 hours until confluent. Cells were then cultured with 1ml of either complete medium, pre-exercise conditioned serum (10% serum with medium) or post-exercise conditioned serum (10% serum with medium) from the same participant for a further 24 hours. Then the supernatant was removed and kept in a separate falcon tube for each well. 500µl of 0.05% trypsin was used to lift cells by incubating for 5 minutes at 37°C. Cell solutions from each well were then placed in falcon tubes containing their previous supernatant and cells were washed with 1ml of PBS followed by 200µl of 1X Annexin V Binding Buffer (ThermoFisher Scientific, prepared from 1 part 5X binding buffer and 4 parts distilled water). Cells were resuspended in 100µl of binding buffer with the addition of 5µl of FITC-annexin V (BioLegend) and 5µl of anti-calreticulin/Alexa-Fluor 647 (Novus). A 15-minute incubation took place at room temperature in the dark.

Following this, 2ml of 1X binding buffer was added to each tube and then centrifuged at 2000 RPM for 5 minutes. The supernatant was removed and cells were placed in 200µl of 1X binding buffer. Cells were incubated on ice for 15 minutes with the addition of 5µl of propidium iodide. Samples were then stored within a 4°C fridge. FACS analysis was performed with the Cytek Aurora Flow Cytometry System within 4 hours.

2.9: Oncolytic viruses (OVs)

2.9.1: Plaque assays

Plaque assays were done to establish the viral titre of the OV HSV1716 from our group stock. 1 x 10^5 Vero cells were seeded into each well of a 12-well plate. Cells were cultured in 1ml of DMEM-F12 (ThermoFisher Scientific) with 10% FBS and 1% penicillin-streptomycin for 24 hours until confluent. Media was then removed and 1ml of warmed PBS was used to wash the wells.

Then HSV1716 was serially diluted ten times by one log each time. 100µl of each dilution was added to the Vero cells in separate wells. 100µl of PBS was added to the 2 final wells to act as controls. The plate was then incubated at 37°C for 2 hours during which the plate was tilted up/down and left/right every 30 minutes.

During this incubation, 4% agarose solution was made by adding 4g agarose to 100mls water. This was then warmed for 1 minute in a microwave and then placed in a 60°C water bath. 20mls of warmed DMEM-F12 was then mixed with 2mls of warmed 4% agarose. 1.5mls of this solution was then added to the wells and allowed to set at room temperature. The plate was then incubated at 37°C for 72 hours.

1ml of 4% PFA was then added to each well and the plate was incubated for a further 2 hours. Then the PFA was aspirated off and agarose plug carefully removed using a spatula. Wells were washed with PBS and enough crystal violet was added to each well to cover the base. Plates were kept at room temperature for around 5 minutes before washing off the crystal violet.

The wells were observed and those that had around 50-100 plaques within the monolayer were identified. The exact number of plaques were counted and the viral titre (plaque forming units (pfu)/ml) identified by the number of plaques (with 100µl of virus) multiplied by 10 multiplied by the dilution factor.

2.9.2: Multiplicity of Infection (MOI) for HSV1716

In order to assess the appropriate MOI required to kill 50% of the cancer cells in vitro and therefore identify the IC50 (half maximal inhibitory concentration) required in further in vitro work, wells were seeded with either 5,000 PANC-1 cells or MSTO-211H cells and incubated for 24 hours at 37°C until confluent. Following the plaque assay, the known HSV1716 titre was serially diluted to create MOI 10, 5, 2.5, 1.25, 0.6 and 0.3. In triplicate, HSV1716 at each MOI was added to both the PANC-1 and MSTO-211H cells and then cultured for a further 24 hours. AlamarBlue® assays were then used at 1, 2, 6, and 7 days to assess cell viability at different HSV1716 MOIs.

2.9.3: Cell viability HSV1716 with complete medium, pre-exercise or post-exercise conditioned serum

To assess the combination of exercise-conditioned serum and the OV HSV1716, viability assays were performed using alamarBlue® on MSTO-211H and PANC-1 cells.

5000 cells were seeded onto wells on a 96-well plate. After incubation for 24 hours and development of a confluent cellular layer, cells were treated as per Figure 2.4.

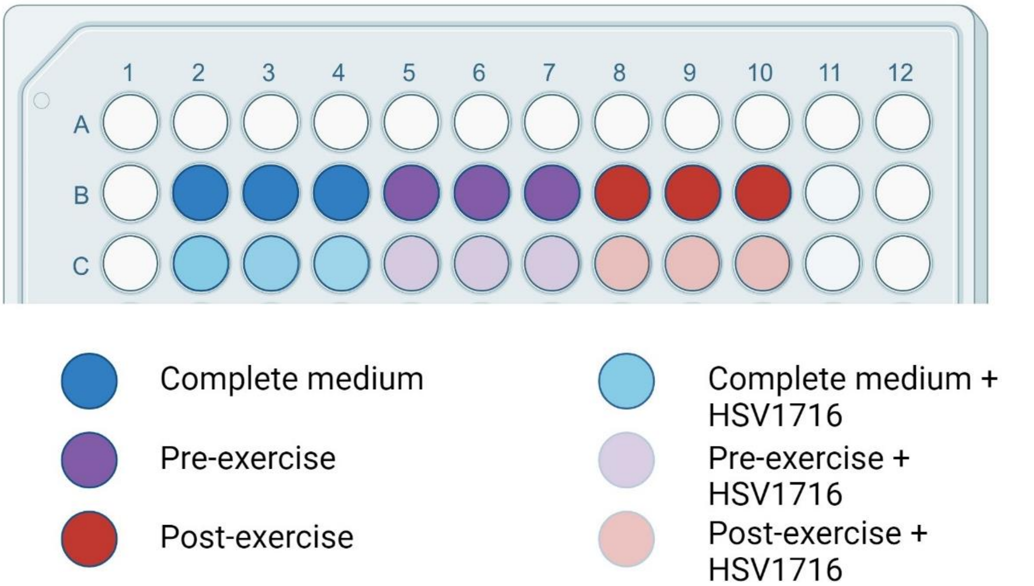


Figure 2.4: Incubating strategy for assessing cell viability using a 96-well plate

MSTO-211H and PANC-1 cells were cultured with complete medium, pre- and post-exercise condition serum with and without HSV1716 at an MOI of 1 in a 96 well plate. Samples were analysed in triplicate and outer wells were avoided.

In total, 100µL of culture medium with or without human serum was added to each well. In wells treated with human serum, 90µl of serum-free medium and 10µl of human serum were added. In wells treated with HSV1716, an MOI 1 was added (hence as the viral titre was $1x10^6$ pfu/ml, 5µl containing 5000 pfu was added to wells with 5000 cells). Each treatment combination was tested in triplicate. This was repeated twice on the same well plate to allow analysis of the two participant's serum. Following a further 24 hours of incubation, 10µl of alamarBlue® was added to each well and incubated for 4 hours before fluorescence was read using the Ensight Multimode Microplate reader. Solutions in the wells were then discarded, wells washed with 100µl of PBS and then re-treated with the same previous conditions in order to perform alamarBlue® assays on day 2, 6 and 7.

2.9.4: HSV infectivity assays

1 x 10^5 PANC-1 cells were seeded in 2mls of DMEM medium into 5 wells of a 6-well plate. 1 x 10^5 Green Fluorescent Protein (GFP) expressing JJN3 myeloma cells (non-adherent) were added to the final well as a positive control.

PANC-1 cells were incubated for 24 hours until confluent. Medium from PANC-1 wells was discarded and wells were washed with 1ml of PBS. GFP expressing

HSV1716 was then added at MOI 1 in 500µl of serum-free medium to 4 wells with PANC-1 cells, the fifth well acting as a negative control with unstained cells, with only 500µl of serum-free medium added. The plate was incubated for 2 hours at 37°C and then a further 2.5mls of complete medium was added to each well.

Following 24 hours, wells were imaged using the GFP filter on the Invitrogen™ EVOS™ M7000 Imaging System (ThermoFisher Scientific). The supernatant was collected from 3 wells containing the PANC-1 cells and PBS was used to wash the wells. The other 2 wells were processed at 48 hours in the same way. 1ml of trypsin used to detach cells with incubation for 5 minutes at 37°C. 2mls of complete medium and the supernatant collected prior were added to each well and these solutions and the JJN3 cell suspension were spun at 1000 RPM for 5 minutes. After discarding the supernatant, 5mls of PBS was used to wash the pellet prior to centrifuging under same conditions. Following this, the supernatant was removed and 400µl of PBS was added to the pellet for resuspension and placed on ICE. Samples were run through the Cytek Aurora Flow Cytometry system immediately and GFP-positive populations were measured.

2.10 In vivo effects of exercise on pancreatic cancer and mesothelioma

Animal procedures and interventions were undertaken in keeping with the UK Animals (Scientific Procedures) Act 1986 and carried out under a UK Home Office approved amendment to PP1099883. 6-8 week old male and female C57BL/6 and BALB/c mice were purchased from Charles River Laboratory and underwent a 7-day acclimatisation period in the Biological Services Unit, University of Sheffield, prior to any intervention (Personal License: Sonal Brindley Hapuarachi, I30565184).

2.10.1: Orthotopic implantation of pancreatic cancer

Surgery for the pilot study was carried out under the supervision of Dr Paris Avgoustou. Surgery for the larger study assessing the combination of exercise and anti-PD1 was carried out by myself, assisted by Dr Hawari Bin Mansoor.

Mice were initially orally administered meloxicam 5mg/kg. Mice were then anaesthetised using inhaled 5% isoflurane. Mice were shaved over 2cm x 2cm area covering the left lateral thoraco-abdominal region. 0.05mg/kg of subcutaneous buprenorphine was administered and mice were placed on a 37°C heating pad. Skin was prepared using 10% povidone iodine solution. Under maintenance 3% isoflurane, 4 x 10^5 Panc02 luciferase-labelled cells were injected in a 20ul matrigel/PBS/cell suspension into the tail of the pancreas, which was identified after gentle lifting of the spleen from the abdominal cavity and securing with a sterile cotton bud. After returning the spleen and pancreas to the abdominal cavity, muscle layers were closed with continuous absorbable 5-0 sutures. Non-absorbable 6-0 interrupted sutures were used to close the skin.

Following surgery, mice recovered within their warmed cage on a 37°C heating pad and observed for 2 hours and reviewed at 4 and 6 hours following surgery.

2.10.2: Orthotopic injection of mesothelioma cells into BALB/c mice

1x10^5 murine mesothelioma AB1-Luciferase cells were injected intraperitoneally into BALB/c mice (n=24). Mice were carefully monitored for any adverse events for 1-2 hours following injection. Due to the high likelihood of malignant ascites, abdominal distension was monitored carefully throughout the study. The mouse was restrained gently by the tail base and the abdomen visualised cranially. When the distended abdomen became wider than the rest of the mouse (equivalent to an 11-day pregnant mouse), the mouse was humanely killed.

2.10.3: Treadmill running

Mice in the treadmill group performed running for 15-20 minutes/day at 12 metres/minute for a minimum of 4 days per week using a modified mouse treadmill (stimulation by paint brush spike).

2.10.4: Horizontal Screen

For the horizontal screen resistance training, the mouse was gently positioned on top of a 29x29cm wired screen. A stop clock was started and the screen was inverted. The mouse would be kept upside down 30cm over a soft surface for 1 minute. If unable to hang upside down, the time taken for the mouse to fall off the screen was documented (55).

The pilot study consisted of 12 C57BL/6 mice in 4 groups (n=3). All mice underwent orthotopic pancreatic cancer implantation on day 0. Treatment included a non-exercise control group and different exercise activities as per Table 2.4a. The larger study included 20 C57BL/6 mice in 4 groups (n=5). The mesothelioma study included 24 BALB/c mice in 4 groups (n=6). Exercising mice underwent a combination of treadmill-running and resistance training and mice were given either anti-PD1 or an isotype IgG2a control as per Table 2.4b. An overview of the latter 2 studies can be found in Figure 2.5.

Table 2.4a: Treatment schedule for the pilot study

C57 BL/6 mice		**Tumour development**			**Treatment**		
Group	**No. mice**	**Cell line**	**Route Admin**	**Volume (µl)**	**Treatment**	**Route Admin of drug**	**Volume of drug (µl)**
1	3	Panc02	Pancreatic injection	20ul	Sedentary	N/A	N/A
2	3				Horizontal screen exercise		
3	3				Treadmill		
4	3				Horizontal screen exercise and treadmill		

Table 2.4b: Treatment schedule for exercise/anti-PD1 study

Mice		**Tumour development**			**Treatment**		
Group	**No. mice**	**Cell line**	**Route Admin**	**Volume (µl)**	**Treat-ment**	**Route Admin of drug**	**Volume of drug (µl)**
1	5	Panc 02	Pancreatic injection	20 µl	Isotype control + no exercise	Intra-peritoneal injection	100 µl
2	5				Isotype control + exercise		

3	5				Anti-PD1 + no exercise		
4	5				Anti-PD1 + exercise		
1	6	AB-1	Intraperitoneal injection	100 µl	Isotype control + no exercise	Intra-peritoneal injection	100 µl
2	6				Isotype control + exercise		
3	6				Anti-PD1 + no exercise		
4	6				Anti-PD1 + exercise		

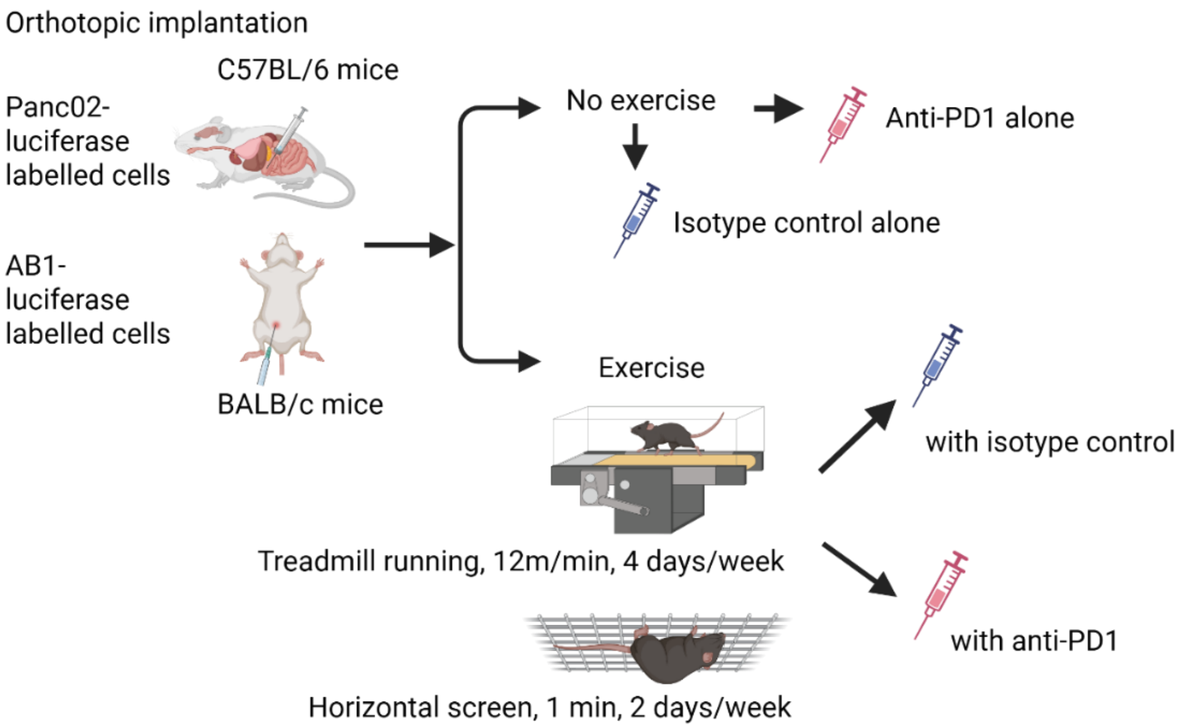


Figure 2.5: Overview of mouse studies

Orthotopic implantation of pancreatic cancer cells (Panc02-luciferase) and mesothelioma cells (AB1-luciferase) were performed surgically into the pancreas of C57BL/6 mice and via intraperitoneal injection in BALB/c mice respectively. The diagrams outline the different intervention arms.

2.10.5: In Vivo Imaging System (IVIS) analysis

Pancreatic cancer and mesothelioma was assessed once a week utilising the in vivo imaging system (IVIS). Mice were anaesthetised with 5% inhaled isoflurane and maintained on 2% isoflurane in the imaging chamber. Mice were injected intraperitoneally with 100µl luciferin (6mg/ml, Abcam) 7 minutes prior to image capture.

2.10.6: Humane endpoints

Mice were weighed daily throughout the duration of the study. Mice were immediately killed by a Schedule 1 method if they showed one or more of the following:

1) Weight loss exceeding 15% compared to age matched controls, weight loss exceeding 10% in those with ascites present.

2) When weight loss was more gradual and within the 15% limit and no other clinical signs were present, animals were monitored and if they either developed other clinical signs or failed to show a weight increase within 48 hours, they were killed immediately.

3) Body condition score of 2. Criteria included marked and persistent piloerection, eating/drinking difficulties, weight loss (see above) and persistent hypothermia up to the end of the day (signs of shivering, changes body posture (eg. hunching) and huddling.

4) Convulsions

5) Increase in abdominal size equivalent to 11-day pregnant mouse.

6) Abdominal rigidity, guarding or pain on palpation

7) Dehydration lasting up to 24 hours (using a skin pinch test)

8) Any single tumour/ sum of multiple tumours that reached the maximum permitted size of 12mm as determined with IVIS.

2.10.7: Mouse dissection and sample preparation

Once animals reached a humane endpoint or the end of the study, a cardiac terminal bleed was performed followed by cervical dislocation. The tumour, spleen, liver and lungs were dissected. The tumour was divided into two, half placed in fresh medium to undergo flow cytometry preparation immediately after. The other half of the tumour along with the spleen, liver and lungs were placed in 4% PFA for 24-48 hours. These tissues were then transferred into 70% ethanol in order to be paraffin wax embedded and sectioned with a slide from each mouse Haematoxylin and Eosin (H&E) stained (see section 2.10.10). Blood samples from terminal bleeds were spun for 10 minutes at 2000 RPM and serum was extracted and stored in the -20°C freezer to allow analysis at a later date.

The flat end of a 10 ml syringe was used to break up the tumour placed on a cell strainer over a 50 ml falcon tube in order to create a single cell solution, whilst continuously being washed through with media. The cell solution was then centrifuged at 1600 RPM for 5 minutes. Following removal of the supernatant, the pellet was washed with 30 ml of medium. Following further centrifuging, an automatic haemocytometer was used to count the cells. $1x10^6$cells were then stained with the antibodies (see Table 5) using the same protocol as described earlier. Samples were stored in PBS overnight following fixation in 4% PFA and analysed using the Cytek Aurora Flow Cytometer the following day.

2.10.8: Flow cytometry analysis for immune cell populations within tumours

Flow cytometry was used to identify and quantify the different immune cells present within the pancreatic cancer and mesothelioma TMEs. The following antibody panel was optimised using single stains as per Table 2.5.

Table 2.5: Optimum anti-mouse antibody-fluorophore concentrations

Antibody-fluorophore conjugate	Amount required (per $1x10^6$ cells)
CD3 – PerCP/CY5.5	0.5µg
Ly6C – AF700	0.125µg
CD11b – PE/ Dazzle 594	0.03µg

CD4 – FITC	0.125µg
NK1.1 - APC	0.125µg
CD25 – BV421	0.0625
CD8 – BV605	0.25µg
CD45 – BV785	0.25µg
Zombie NIR dye	1µl

2.10.9: H&E analysis

Paraffin-wax embedding and sectioning using Leica RM2135 were performed by Ms Maggie Glover. In brief, paraffin wax-embedded slides were fully submerged in xylene for 10 minutes, and transferred to a further container of xylene for another 5 minutes. Hydration of the slides was performed by initially submerging in 100% ethanol for 5 minutes, another container of 100% ethanol for 3 minutes and then in 95%, 90% and 70% Industrial Methylated Spirits (IMS) sequentially, each for 3 minutes. Slides were then washed under running water for 5 minutes. Slides were added to Haematoxyllin solution for 1 minute and then placed in running water until the water became clear. Slides were then dehydrated by submerging in 70% and then 90% ethanol sequentially for 3 minutes each.

Slides were then added into eosin in 95% ethanol for 30 seconds. They were then placed in 100% ethanol followed by 100% H&E for 3 minutes. DPX mountant was used to mount the coverslips.

H&E slides were scanned using the Epredia Panoramic 250 Flash III Slide Scanner and viewed on SlideViewer®. Metastases from liver sections were counted and percentage area of liver affected by metastases also measured. Tumour histology was reviewed, and percentage area of necrosis was measured. Statistical significance was determined and graphs were generated using GraphPad Prism.

2.10.10: Immunofluorescent staining

Paraffin wax-embedded slides were de-waxed in the same process described above (see section 2.10.10). 10 mls of Dako Target Retrieval Solution X10, Citrate pH 6 was added to 90 mls of deionised water and slides were directly transferred from water into this Dako solution. Slides were then heated in this solution in an antigen retrieval pressure cooker for 2 hours.

3 Litres of PBS-Tween20 (PBST) was made up using 30 PBS tablets, 3 litres of deionised water and 3 mls of Tween-20. Following the 2-hour heating incubation, slides were washed in 400 mls of PBST for 5 minutes twice. A wax pen was used to demarcate the tissue on the slide. Following the addition of 2.5% horse serum and 10% Murine FcR blocking solution diluted in PBST, incubation of the slides took place at room temperature for 30 minutes. Excess blocking solution was tapped off and conjugated antibodies at 1:100 dilutions in PBST (see Table 2.6) were added to the slides and incubated for 40 minutes at room temperature. Following this, PBST was used to wash the slides 3 times and 1 drop of ProLong Gold Antifade mountant with 4',6-diamidino-2-phenylindole (DAPI) was added prior to coverslip addition.

Slides were stored within foil at 4°C for 24 hours and then visualised using the Zeiss LSM 980 Airyscan Confocal microscope. Images were analysed using Fiji ImageJ.

Table 2.6: List of conjugated antibody groups used for immunofluorescent staining

Conjugated antibodies
FITC-CD8, PE-NK1.1, AF647-Granzyme B
PE-PD1, FITC-CD8
F4/80 – PE, MRC1-AF647

2.11: Statistical analyses

GraphPad Prism v.8 was used to ascertain statistical significant differences and provide graphs. Data are expressed as median values with standard deviations. Due to the sample sizes, non-parametric tests were used to analyse the data unless data was confirmed to follow a normal distribution using Shapiro-Wilk tests when parametric tests were performed. Wilcoxon signed-rank tests were used to assess significant differences between paired data, when pairing was not possible, data were assessed using Mann-Whitney tests. To determine statistically significant differences between 2 or more groups, one-way ANOVA (Kruskal-Wallis) tests were performed. For proven normally distributed data, ordinary one-way ANOVA and unpaired t-tests were used. A p value of <0.05 was deemed statistically significant.

Chapter 3

Effects of exercise on immunity and in vitro effects of exercise on pancreatic cancer and mesothelioma cell lines

3.1 Introduction

Exercise induced leucocytosis is an established phenomenon with varying systemic immune responses dependant on frequency, intensity, type and duration of exercise. Previous research has demonstrated a favourable systemic anti-cancer immune profile, with raised cytotoxic immune populations such as NK cells and myokine release seen with moderate intensity exercise with resistance training (18,56). Stromberg et al demonstrated high intensity sprinting significantly increased both amount and cytotoxicity of CD8 T cells (57). The IFNγ-mediated cell destruction was non-specific as demonstrated by reduced HEK293T (immortalised embryonic kidney cell) viability. A breast cancer mouse model demonstrated correlation between significant increases in systemic white bloods cells, including neutrophils and monocytes with higher concentration of cytotoxic CD8 T cells and reduced regulatory T cells (Tregs) within the TME with wheel running (58). This highlights tumours are able to benefit from systemic proliferation of effector immune cells and reduction of immune tolerant cells, potentially by normalisation of tumour vasculature and improved delivery (34).

General deterioration of the functionality of the immune system occurs with the aging process and patients affected by pancreatic cancer tend to be >60 years old with the highest incidence of mesothelioma in adults >80 years old (59–61). Therefore, an area of concern would be the potential lack of efficacy of exercise in immune modulation in the elderly. Ruple et al extensively immune profiled blood from two groups of participants based on age (average age 23 vs 65 years old) after a high intensity aerobic and resistance exercise (62). Their study demonstrated that exercise enhanced NK cell activity irrespective of age with significant increases in IL-6, a potent myokine implicated in anti-cancer immunity, in both groups, although a more blunted response was seen in the older adults (62). Interestingly, gene profiling before and after the exercise intervention revealed a significant reduction in the age-related differences in genes involved in immune functioning suggesting that exercise may be able to combat issues with immunosenescence in the elderly (62).

Exploring exercise-induced benefits within a cancer population, the SPRINT trial revealed a 6-week combination moderate-high intensity aerobic and resistance training programme alongside neoadjuvant treatment in early breast cancer led to increased CD56+ cell intratumoural infiltration implicating enhanced NK/CD8 T cells within the TME (63). Studies have highlighted the improved prognoses seen in a range of tumours with higher levels of these cytotoxic immune cells (64). Therefore, exercise could be an effective cancer treatment by modulating systemic and intratumoural immunity.

For ICIs to be effective, the necessary immune components, such as cytotoxic CD8 T cells, need to be present within the TME, which are lacking in these cold tumours. Exercise could be the pivotal step in reprogramming the immune climate allowing immune checkpoint inhibitors to work better in mesothelioma and pancreatic cancer. This in turn could broaden the treatment options for these cancers, improving survival outcomes.

The direct impact of aerobic exercise-conditioned serum on pancreatic cancer and mesothelioma cell lines is yet to be explored. Schwappacher et al reviewed the effects of resistance exercise simulation with whole body electro-myosimulation (WB-EMS) on pancreatic cancer patients, where electrical impulses were used to stimulate contraction of large muscle groups throughout the body (65). They demonstrated that serum from these patients had the ability to reduce pancreatic cancer cell growth, increase apoptosis and inhibit migration in vitro. WB-EMS could potentially be a feasible and practical method to simulate exercise in patients that may be too frail/ unwell to undertake exercise activities. However, these patients would unlikely be candidates for systemic anti-cancer treatment and therefore, carry the poorest prognoses. This chapter aims to demonstrate the anti-cancer benefits of physical aerobic exercise, which in turn will allow patients to reap the extra benefits on cardio-respiratory and musculoskeletal health that exercise has been shown to provide in the cancer population (11).

The effect of exercise on immune tolerant cells such as Tregs and MDSCs are less well described. Wang et al (3) demonstrated enhanced Treg activity with high intensity exercise training within a non-cancer mouse model. If these findings correlate with a cancer model, the potential benefits of enhanced cytotoxicity could be counteracted. However, other studies have demonstrated a beneficial reduction in Tregs systemically and intratumourally with moderate-intensity exercise (58,66).

There is a clear unmet clinical need to improve treatment in the poorer prognostic tumours that lie on the cold end of the spectrum, where immunotherapy has no (pancreatic cancer) or little (mesothelioma) effect. This chapter aims to assess the effect of exercise on these cancers in vitro.

3.2: Hypothesis

1) A single bout of high intensity interval aerobic exercise will lead to enhanced effector immune cells and reduced immune tolerant cells within the blood of healthy volunteers.
2) Exercise will enhance pro-inflammatory, anti- cancer cytokines leading to reduced pancreatic cancer and mesothelioma cell viability and migration and increased apoptosis
3) Exercise-conditioned serum will work synergistically with OV (a form of immunotherapy) to enhance cytotoxicity.

3.3: Aims and Objectives

1. Detail the systemic immune response to high intensity interval aerobic exercise in healthy volunteers, including cytotoxic NK/ CD8 T cells and Tregs.
2. Quantify cytokines from pre- and post-exercise conditioned serum using cytokine bead arrays
3. Assess the effect of exercise-conditioned serum on pancreatic cancer and mesothelioma cells compared to matched pre-exercise serum by measuring cell viability, migration and apoptotic activity
4. Investigate the infectivity of pancreatic cancer and mesothelioma cells with the OV, HSV1716, and assess viability of these cells when cultured with a combination of exercise-conditioned serum and HSV1716

3.4: Results

3.4.1: Baseline Demographics

22 healthy volunteers were recruited to the study from the University of Sheffield, including staff and students. The baseline demographics are shown in Table 3.1. Data from both pre- and post-exercise conditioned blood were available for 20

participants, one participant was excluded due to insufficient blood samples and one was excluded due to a technical error with the flow cytometer. We were unable to perform analysis of CD4+ cell counts on one participant due to lack of availability of the CD4-BV510 conjugate. Ages of participants ranged from 23 to 62 years old and included 45% (10) male and 55% (12) female participants with the majority having no co-morbidities (18, 82%) and 91% were non-smokers (20). Participants with varying baseline fitness were included, although all participants performed some form of regular exercise with 68% (15) performing regular high intensity exercise during the week. Flow cytometry with a panel of immune markers was carried out on collected blood samples.

Table 3.1: Baseline characteristics of the healthy volunteers

	Number of participants
Age (years)	
23-25	2
26-30	7
31-35	7
36-40	0
41-45	2
46-50	1
51-55	1
56-60	1
61-65	1
Gender	
Male	10
Female	12
Co-morbidities	
0	18
1	2
2 or more	2
Baseline fitness	
No regular exercise	0
1-2 week moderate intensity	2
3-4 week moderate intensity	2
5-7 week moderate intensity	3
1-2 week high intensity	4
3-4 week high intensity	5

5-7 week high intensity	6
Smoker	
Y	2
N	20

3.4.2: Flow Cytometric analysis of bloods samples before and after exercise

Figure 3.1 demonstrates the gating strategy adopted to quantify the following leucocyte subpopulations; CD45 (general leucocyte marker), CD3 (T cells), CD4 (T helper cells), CD8 (cytotoxic T cells), CD20 (B cells), CD14 (monocytes) and CD56 (NK cells). CD25, CD69 and HLA-DR were also utilised to assess T cell activation.

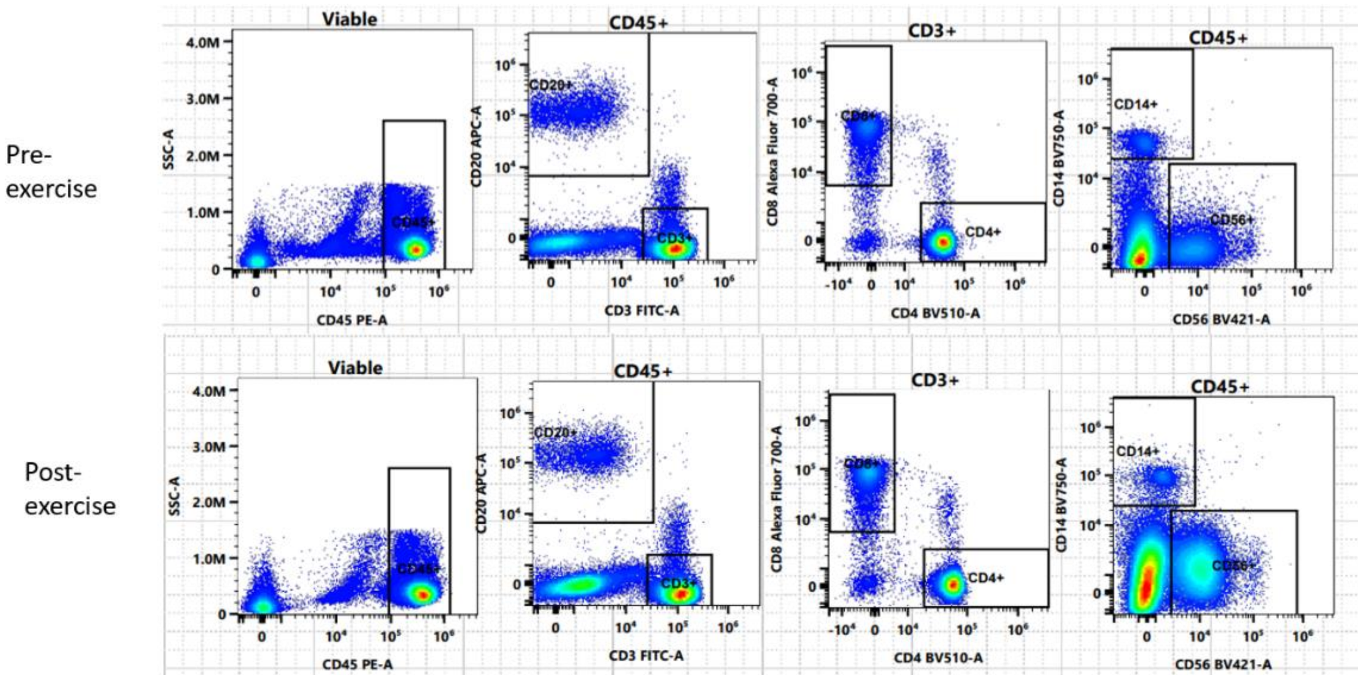


Figure 3.5: Flow cytometry gating strategy comparing pre- and post- exercise conditioned whole blood from one participant

Representative dot plots of viable CD45 (general leucocyte marker) cells were selected using the Cytek Aurora Flow Cytometry System and separated into CD3+ cells (T cells) and CD20+ cells (B cells) as well as CD56+ (NK cells) and CD14+ cells (monocytes). The CD3+ T cell population was used to identify CD4+ (T helper) and CD8+ (cytotoxic) T cells. T cell activation markers (CD25, CD69 and HLA-DR) were measured within the CD4+ and CD8+ T cell populations. The top panel illustrates the leucocyte subpopulations within the pre-exercise conditioned blood of one participant, whilst the bottom panel shows these cells in the blood of the same participant post exercise.

Blood samples pre and post exercise were stained with a panel of immune markers to assess the impact of exercise. Interestingly, Figure 3.2 illustrates the significant changes seen in NK (CD56) cells before and after the acute high intensity exercise in 20 participants. A significant 32.6% rise in CD56+ NK cells was observed post aerobic exercise (p<0.0001, 95% CI 23.0 to 42.2%).

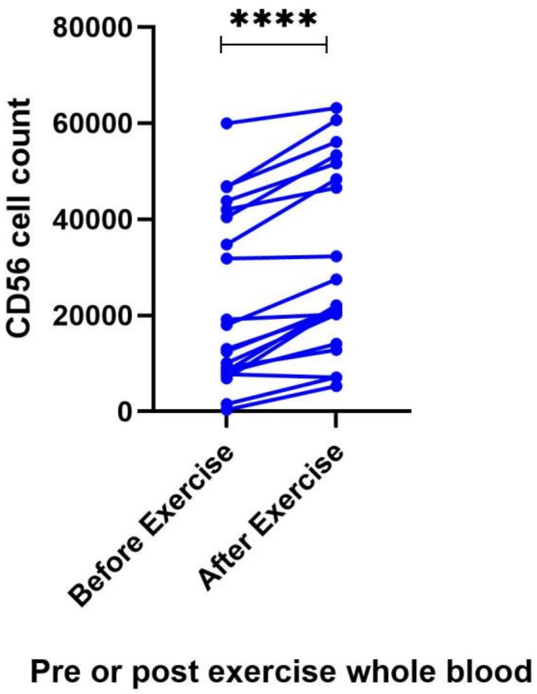


Figure 3.2: High intensity exercise increases Natural Killer (NK) cell populations

Before and after graph demonstrating CD56 differences pre and post exercise in 20 participants who undertook a high intensity interval aerobic exercise activity. Blood samples were taken prior to and immediately after exercise. Whole blood samples were stained with CD56-BV421 prior to red cell lysis and fixation with 1% PFA. CD56 cell count was quantified using Cytek Aurora Flow Cytometry System. Data were analysed with Wilcoxon signed-rank tests using GraphPad Prism 8.0.2, where ****p<0.0001.

Interestingly, a significant decrease in CD4 T cells occurred following aerobic exercise (28.5%, p<0.0001 95% CI 20.4 to 36.7%) (Figure 3.3). We, therefore, investigated if this reduction was seen in the regulatory subset of CD4 T cells as these play a role in immune tolerance and are implicated in tumour progression and spread. Shown in Figure 3.4a, CD4+CD25+ cells (Tregs) also reduced significantly post exercise (31.0% reduction, p<0.0001 95% CI 13.7 – 48.3%) and a 51.7% reduction (p=0.0017 95% CI 15.3 – 86.7%) was seen in CD4+HLA-DR+ cells (general activation marker) following exercise (Figure 3.4b). CD4+CD69+ cells appear to drop by 25.7% however this did not reach statistical significance (p=0.0919 95% CI -21.3 – 72.8%).

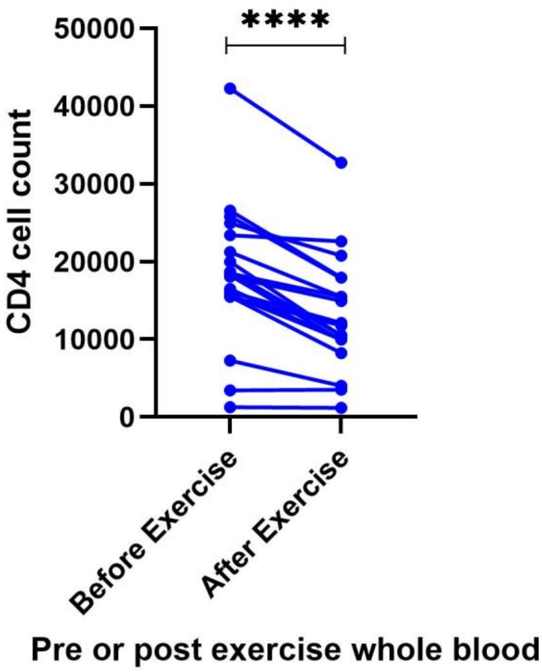


Figure 3.3: High intensity exercise reduces T helper cell populations

Before and after graph demonstrating CD4 (T helper) cell count differences pre and post exercise in each of the 19 participants who underwent a high intensity interval aerobic exercise activity. Statistically significant differences in CD4 (T helper cells) were seen. Blood samples were taken prior to and immediately after exercise. Whole blood samples were stained with CD4-BV510 prior to red cell lysis and fixation with 1% PFA. CD4 cell count was quantified using Cytek Aurora Flow Cytometry System. Data were analysed with Wilcoxon signed-rank tests using GraphPad Prism 8.0.2, where ****p<0.0001.

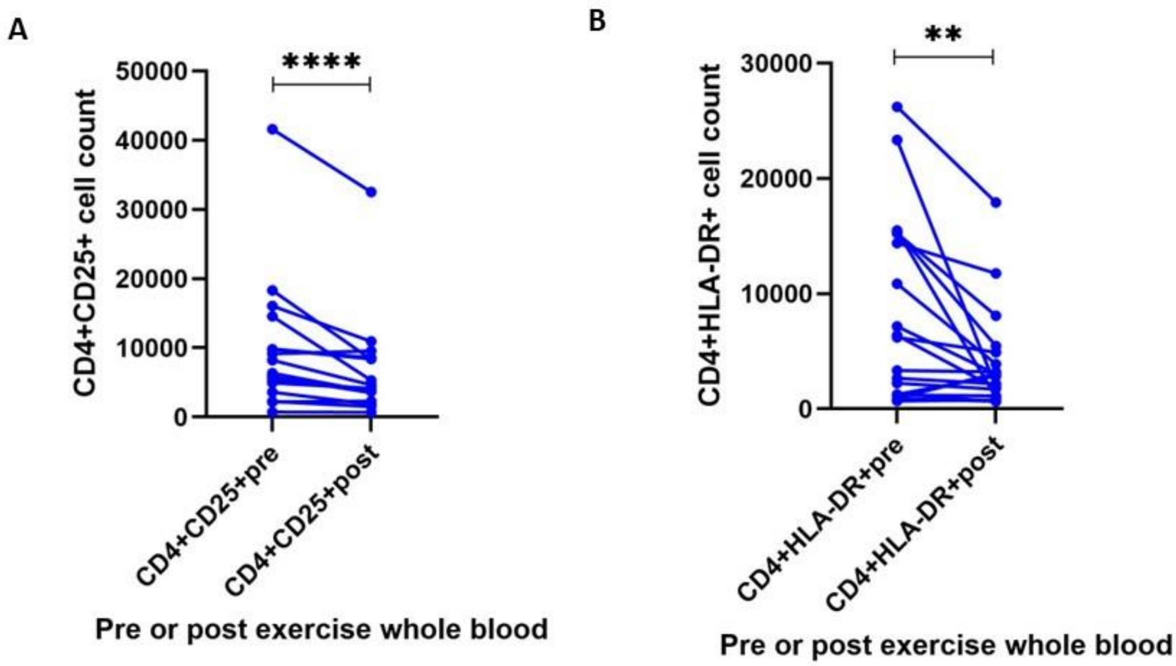


Figure 3.4: High intensity exercise reduces regulatory T cell populations

Before and after graphs demonstrating statistically significant differences in **A.** CD4+CD25+ (Tregs) and **B.** CD4+HLA-DR+ (activated CD4 T cells) cell count following aerobic exercise. 19 participants underwent a high intensity interval aerobic exercise activity. Blood samples were taken prior to and immediately after exercise. Whole blood samples were stained with CD4-BV510, CD25-PEFire700 and HLA-DR-PECy7 prior to red cell lysis and fixation with 1% PFA. CD4, CD4+CD25+ and CD4+HLA-DR+ cell counts were quantified using Cytek Aurora Flow Cytometry System. Data were analysed with Wilcoxon signed-rank tests using GraphPad Prism 8.0.2, where **p<0.01, ****p<0.0001.

Next, we assessed, how exercise affects CD14 cells (monocytes). Monocytes play a key role in achieving homeostasis within the innate immune system and are precursors to macrophages, which are implicated in both pro- and anti-inflammatory pathways (67). As shown in figure 3.5a, these cells were reduced by 17.9% with exercise (p=0.0041 95% CI -0.98 – 44.9%).

However, flow cytometry did not demonstrate significant changes in CD8 cells (Figure 3.5b) immediately following exercise (0.66% increase, p=0.7285) or CD20+ B cells (0.70% increase, p=0.0955), as illustrated in Figure 3.5c.

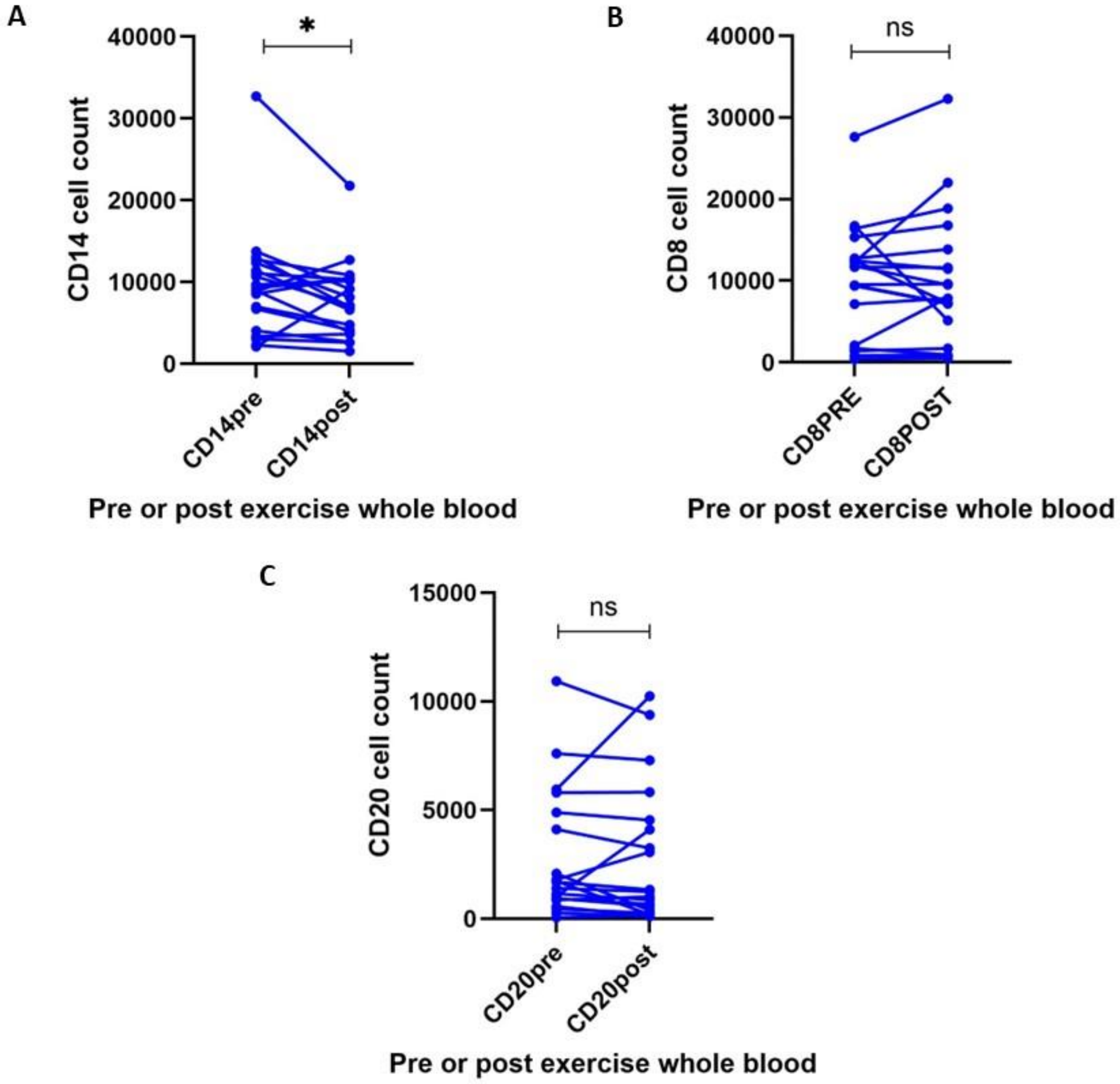


Figure 3.5: High intensity exercise reduces monocyte populations but does not immediately affect cytotoxic T cells or B cells

Before and after graphs demonstrating differences in **A.** CD14, **B.** CD8 and **C.** CD20 cell count following aerobic exercise. 20 participants underwent a single bout of high intensity interval aerobic exercise. Blood samples were taken prior to and immediately after exercise. Whole blood samples were stained with CD14-BV750, CD8-AF700 and CD20-APC prior to red cell lysis and fixation with 1% PFA. CD4, CD4+CD25+ and CD4+HLA-DR+ cell counts were quantified using Cytek Aurora Flow Cytometry System. Data were analysed with Wilcoxon signed-rank tests using GraphPad Prism 8.0.2, where *p<0.05.

Subgroup analysis of participants that undertook regular high intensity exercise at baseline compared to moderate intensity, showed a trend towards a greater release of CD56+ NK cells (Figure 3.6a) and larger reduction in CD4+CD25+ T cells (Figure 3.6b), although these did not reach statistical significance (p=0.1786 and p=0.4998 respectively). No significant differences in any cell count changes were observed between male and female participants. Age did not affect the changes seen in CD56 NK cells with exercise, however CD4 and CD4+CD25+ cell reduction was significantly greater in those under 35 years old compared to those 35 years and over (p=0.0130 and p=0.0060 respectively) as illustrated in Figures 3.6c and d.

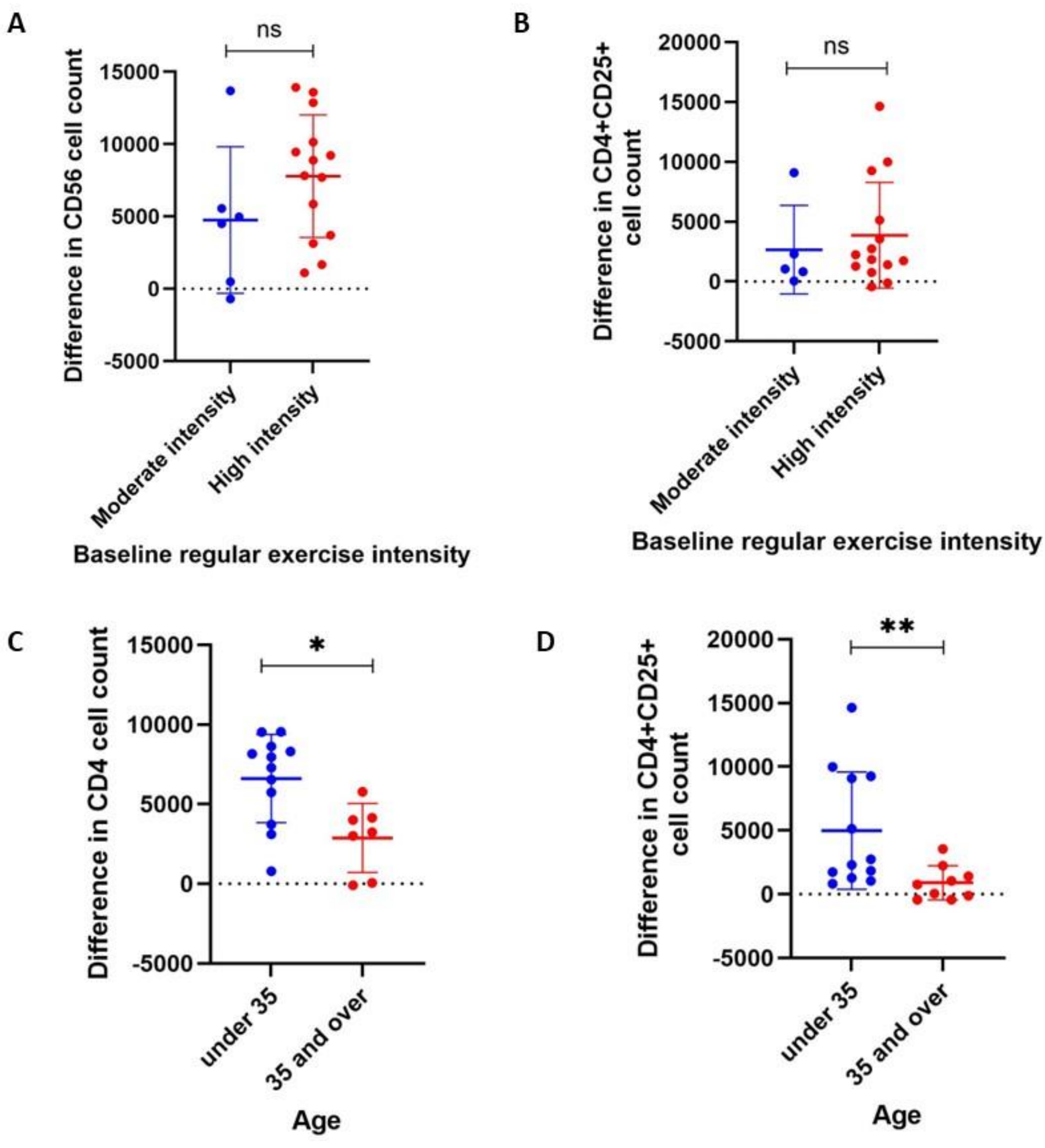


Figure 3.6: Exercise-induced T helper cell and Treg decreases are dependent on age

Scatter plot graphs demonstrating exercise-induced changes in **A.** NK cells (CD56) depending on baseline fitness and changes in **B.** T regulatory cells (CD4+CD25+ T cells) depending on baseline fitness. Fast walking, jogging and cycling at a gentle pace were defined as moderate intensity, whilst running, cycling at a fast pace and intense gym exercises were classed as high intensity. **C.** Illustrates the differences seen in exercise-induced T helper cell (CD4) reduction depending on age. **D.** Illustrates Treg (CD4+CD25+ T cells) decreases depending on age. Cell counts were quantified using the Cytek Aurora Flow Cytometry System and data were analysed with Mann-Whitney tests using GraphPad Prism 8.0.2, where *p<0.05, **p<0.01.

3.4.3: Serum toxicity from healthy volunteers on human mesothelioma and pancreatic cancer cells

Serum from healthy volunteers significantly inhibited mesothelioma and pancreatic cancer cell viability compared to complete medium at different time points (Figures 3.7-3.8) indicating the presence of anti-proliferative and apoptotic factors within the serum. Significantly reduced MSTO-211H cell viability compared to complete medium was seen at 24 hours (pre-exercise: 58% reduction p=0.01, post-exercise: 50% reduction p=0.0128) and 48 hours (pre-exercise: 43% reduction p=0.0009, post-exercise: 40% reduction p=0.0005). At 72 hours, differences in MSTO-211H cell viability were not deemed statistically significant (complete medium vs pre-exercise: 36% reduction p=0.2729, complete medium vs post-exercise: 11% reduction p=0.4404). In a similar manner, significantly reduced PANC-1 cell viability compared to complete medium was seen at 48 hours (pre-exercise: 51.7% reduction p=0.0187, post-exercise 51.1% reduction p=0.0244) and 72 hours (post-exercise: 61.2% reduction p=0.0499).

With both PANC-1 and MSTO-211H cells, no significant differences in viability were seen with pre- and post-exercise conditioned serum at any time points.

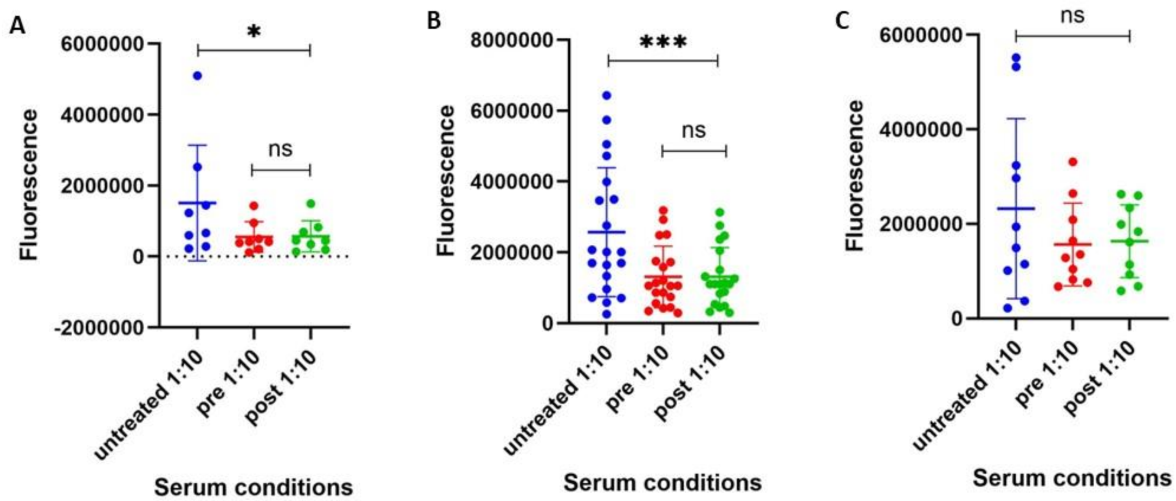


Figure 3.7: MSTO-211H cell viability is significantly reduced with human serum

Cell viability of MSTO cells at **A.** 24, **B.** 48 and **C.** 72 hours cultured with either complete medium (10% FBS), 10% pre-exercise or 10% post-exercise conditioned serum with addition of alamarBlue®. Data were analysed in GraphPad prism 8.0.2 and are expressed as the median and standard deviation (SD) of n=8 (24 hours), n=20 (48 hours) and n=10 (72 hours) independent experiments. Mann-Whitney and Wilcoxon signed-rank tests were used to ascertain statistical significance. Significantly reduced MSTO-211H cell viability compared to complete medium was seen at 24 hours and 48 hours, where *p<0.05, ***p<0.001.

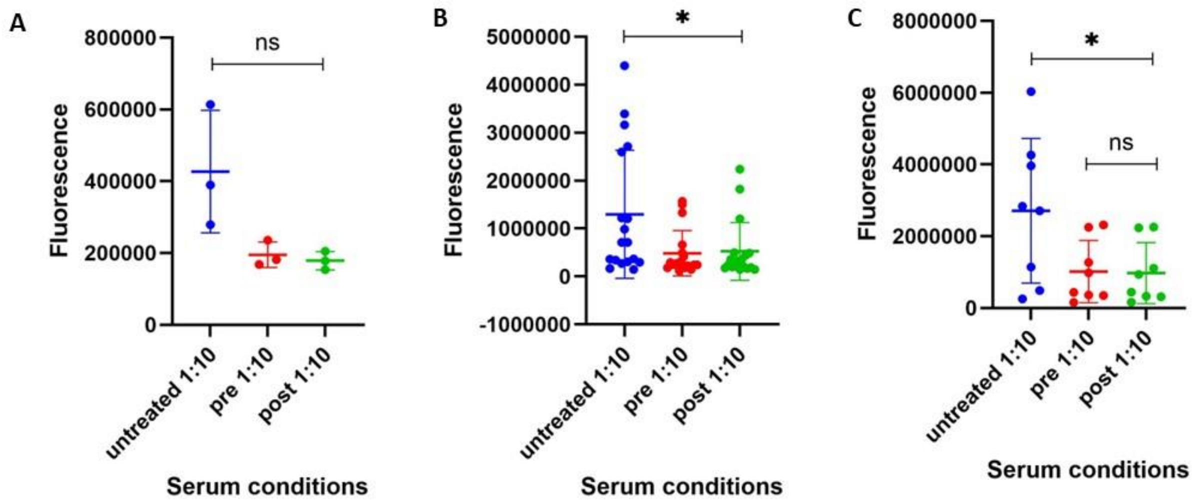


Figure 3.8: PANC-1 cell viability is significantly reduced with human serum

Cell viability of PANC-1 cells determined by alamarBlue® at **A.** 24, **B.** 48 and **C.** 72 hours cultured with either complete medium (10% FBS), 10% pre-exercise or 10% post-exercise conditioned serum. Data were analysed in GraphPad prism 8.0.2 and are expressed as the median and SD of n=3 (24 hours), n=18 (48 hours) and n=7 (72 hours) independent experiments Mann-Whitney and Wilcoxon signed-rank tests were used to ascertain statistical significance. Significantly reduced PANC-1 cell viability compared to complete medium was seen at 24, 48 and 72 hours, where *p<0.05.

3.4.4: Cytokine bead arrays were performed on pre- and post-exercise conditioned serum

Next, we assessed changes in cytokine expression pre and post exercise using cytokine bead arrays analysed on the LSR II Flow Cytometer. Median fluorescence for all cytokines in pre- and post- exercise serum was similar to the 0 pg/ml standard of each cytokine. This indicates that none of these cytokines were detected in any of the participant samples.

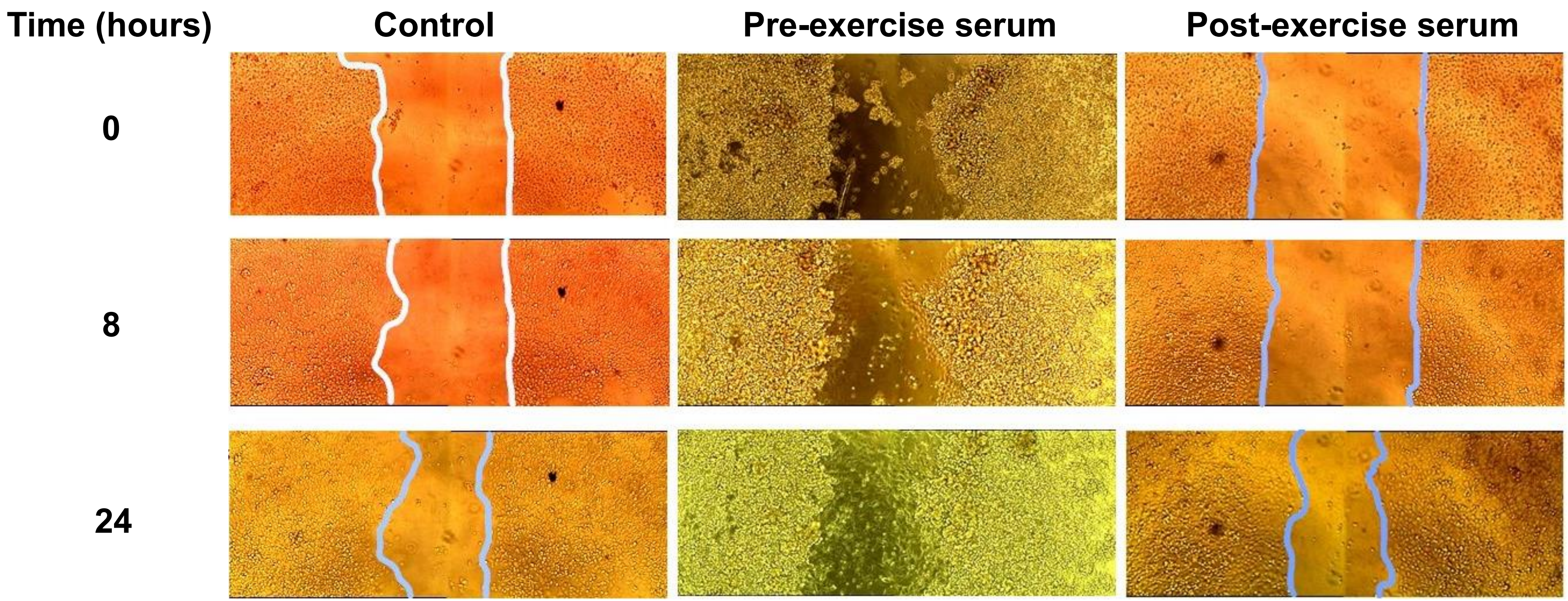
3.4.5: Exercise conditioned serum delays *in vitro* migration of pancreatic cancer cells

5 x 10^5 PANC-1 cells were seeded in 48 well plates until confluency. Cells were then incubated with mitomycin C, an alkylating agent used to prevent cell proliferation, for 90 minutes. Following this, scratches were performed using a 200µl pipette tip, and

cells were cultured in either complete medium, 10% pre-exercise conditioned serum or 10% post-exercise conditioned serum. Images were taken at regular time points using the Invitrogen™ EVOS™ M7000 Imaging System (ThermoFisher Scientific) as shown in Figure 3.9a. Data are only shown for PANC-1 cells due to the inability of MSTO cells to maintain attachment during the scratch assay process despite attempts at addressing issues at each step.

PANC-1 cells demonstrated delayed wound closure on scratch assays when cultured with exercise conditioned compared to pre-exercise serum ($p=0.0313$) as demonstrated in Figure 3.9b. This indicates that high intensity aerobic exercise inhibits the migratory potential of pancreatic cancer cells. Wound closure was significantly delayed when PANC-1 cells were cultured with complete medium compared to pre-exercise conditioned serum and post-exercise conditioned serum ($p=0.0238$ for both).

A


Time (hours)
Control
Pre-exercise serum
Post-exercise serum
0
8
24

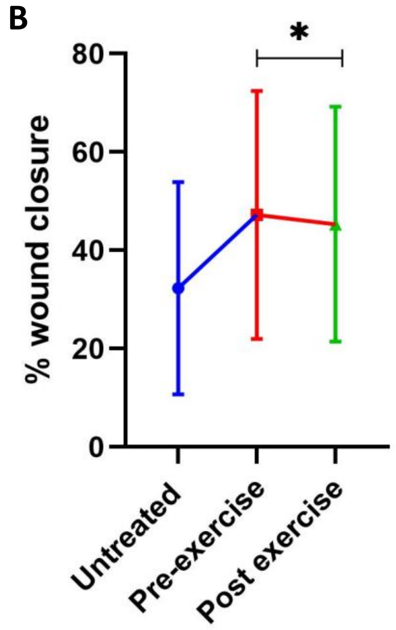


Figure 3.9: PANC-1 cell migration is significantly reduced with exercise-conditioned serum

A. Scratch assays of PANC-1 cells at 0, 8 and 24 hours. **B.** Line graph demonstrating percentage wound closure at selected time points between 0-50 hours of scratch. PANC-1 cells were seeded on a 48-well plate until confluent, treated with 1.3 µg/ml of mitomycin C for 90 minutes to prevent proliferation. Scratches were created using a 200µl pipette tip with the end cut off. Cells were treated with 300 µl of complete growth medium or with 10% of pre- or post-exercise conditioned serum. Images were taken on Invitrogen™ EVOS™ M7000 Imaging System (ThermoFisher Scientific). Data were analysed with Wilcoxon signed-rank and Mann-Whitney tests in GraphPad prism 8.0.2 for n=15 independent experiments.

3.4.6: Exercise conditioned serum delays *in vitro* migration of mesothelioma cells

1x10^5 MSTO-211H cells in 100µl of complete RPMI medium were labelled with Vybrant DiD Cell-Labelling solution and seeded on 3.0µm pore cell culture inserts and placed in a 24-well plate for a 10-minute incubation period. Wells were filled with either complete media (control) or media with pre-exercise or post-exercise conditioned serum. After removal of cells from the apical membrane, and fixing migrated cells on the basal membrane, fluorescent images were taken (Figures 3.10a-c). A significant reduction in mesothelioma cell migration was observed with exercise conditioned serum compared to pre-exercise serum (p=0.0249) (Figure 3.10d).

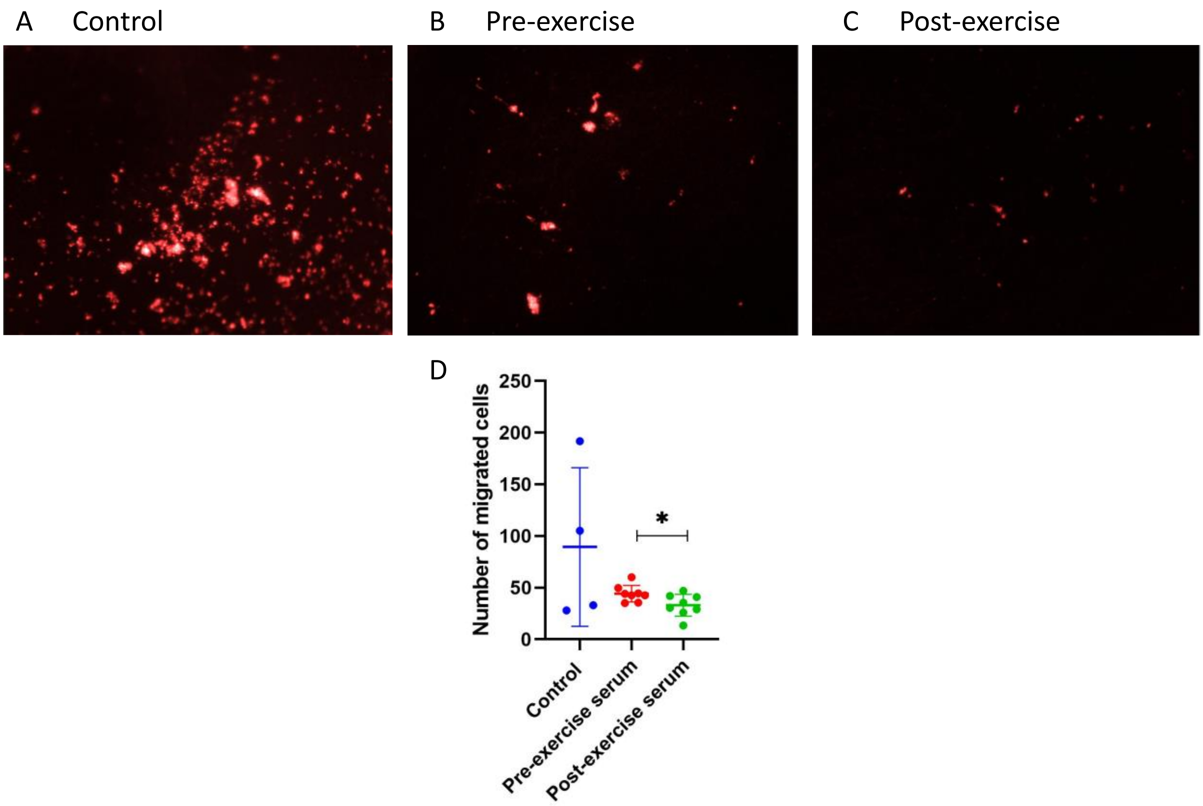


Figure 3.10: MSTO-211H cell migration is significantly reduced with exercise conditioned serum

Transwell migration assay of MSTO-211H mesothelioma cells. 1x10^5 MSTO-211H cells in 100µl of complete RPMI medium stained with DID were seeded on 3.0µm pore cell culture inserts and placed in a 24 well plate. After 10 minutes of incubation at 37°C, wells for filled with **A.** 600µl of complete medium (control), or **B.** 540µl of serum-free medium with 60µl of pre- or **C.** Post-exercise conditioned serum (n=8). Plates were incubated for 2 hours, inserts removed, and cotton-tipped applicators used to remove non-migrated sides from apical membrane. Migrated cells on the basal surface of the membrane were fixed with 4% PFA. Images of basal membrane taken using Invitrogen™ EVOS™ M7000 Imaging System (ThermoFisher Scientific). Data were analysed with Wilcoxon signed-rank tests**,** where *$p<0.05$. **D.** Demonstrates significant reduction in migrated cells with post-exercise conditioned serum.

3.4.7: Exercise-conditioned serum significantly enhances caspase 3/7 activity when cultured with pancreatic cancer and mesothelioma cells

The potential for both aerobic exercise and resistance training to promote myokine secretion has previously been discussed. Aoi et al (28) demonstrate the myokine SPARC's specific ability to induce tumour apoptosis within a mouse colorectal cancer model. This effect was echoed when the addition of the OSM myokine to MCF-7 breast cancer cells led to increased annexin activity, a marker of early apoptosis (68). Furthermore, irisin has been shown to enhance the apoptotic effects of chemotherapy in pancreatic cancer demonstrated by elevated deoxyribonucleic acid (DNA) degradation and raised cleaved poly (ADP-ribose) polymerase (PARP) and caspase 3 levels (69).

In order to assess the apoptotic effect of exercise conditioned serum on pancreatic cancer, caspase 3/7, annexin and calreticulin assays were performed.

Although no significant differences in cell viability were seen between pre- and post-exercise conditioned serum in both cancer cell lines, migration was delayed with exercise-conditioned serum, indicating the presence of anti-migratory factors present within the exercising serum. Apoptotic assays were used to determine the presence of pro-apoptotic factors which would support the scratch assay data. Significantly increased caspase 3/7 levels were observed when PANC-1 cells (16.5% increase, $p=0.0099$, Figure 3.11a) and MSTO-211H cells (6.67% increase, $p=0.0313$, Figure 3.11b) were cultured with post-exercise conditioned serum compared to pre-exercise conditioned serum. Fluorescent images were taken demonstrating the significant caspase 3/7 activity within the cells cultured in exercise-conditioned serum (Figure 3.11c).

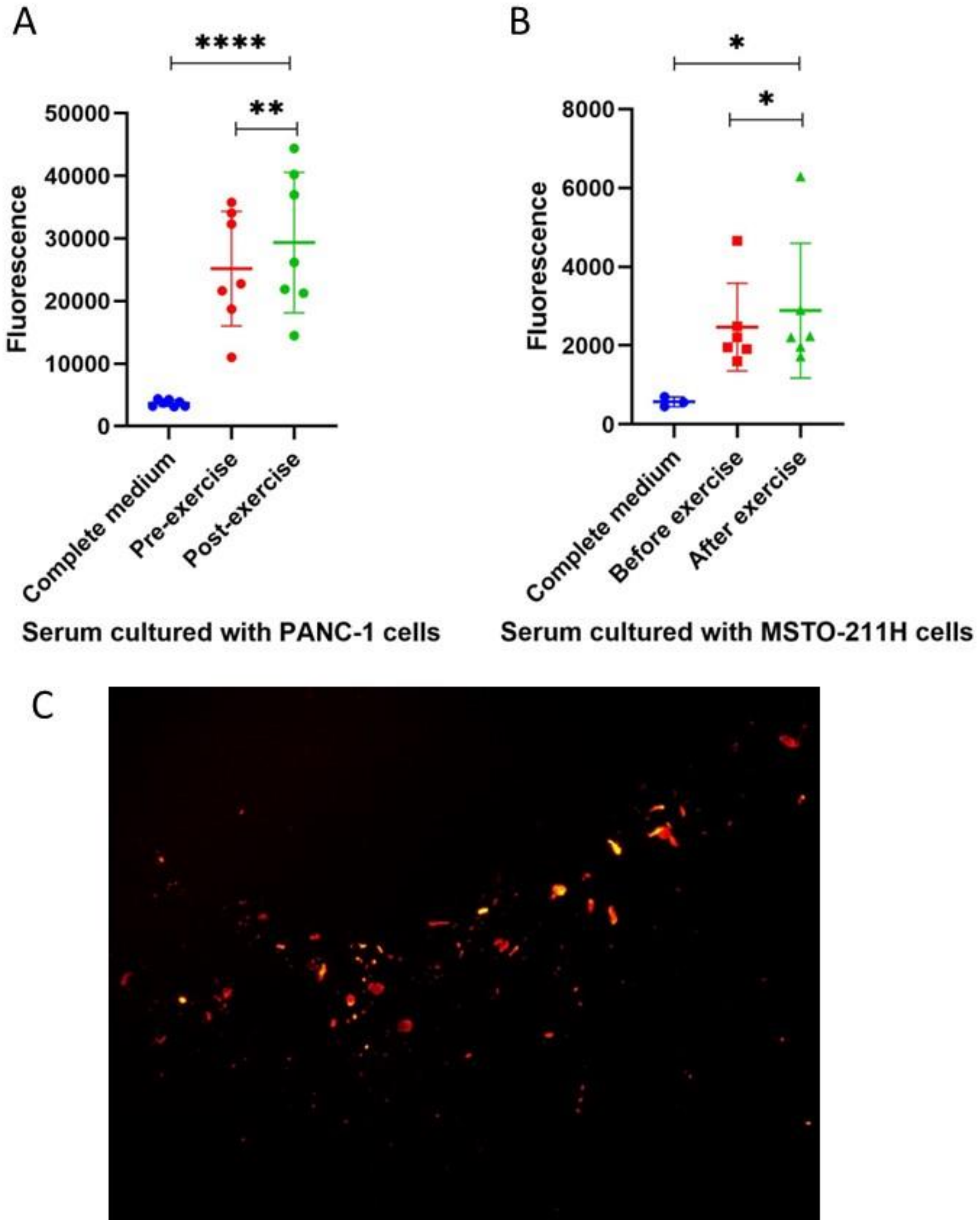


Figure 3.11: Exercise-conditioned serum induces increased apoptotic activity in PANC-1 and MSTO-211H cells

PANC-1 (n=7) and MSTO-211H cells (n=6) were cultured with either complete medium, pre-exercise or exercise conditioned serum for 24 hours. CellEvent™ Caspase-3/7 Red Detection Reagent was incubated with cells for 1 hour. Fluorescence was read using the Ensight Multimode Microplate reader. Significantly increased caspase 3/7 levels were demonstrated when **A.** PANC-1 cells and **B.** MSTO-211H cells were cultured with post-exercise compared to pre-exercise conditioned serum. **C.** Representative image demonstrates the fluorescent signal seen with caspase3/7 activity in MSTO-211H cells cultured with exercise-conditioned serum, using the EVOS fluorescent microscope. Data were analysed in GraphPad prism 8.0.2 and paired t tests were used to ascertain significance if Shapiro-Wilk tests confirmed normal distribution of the data. Otherwise, Wilcoxon signed-rank tests were used, where *p<0.05, **p<0.01 and ****p<0.0001.

3.4.8: Annexin and Calreticulin assays

No statistically significant differences in annexin (Figure 3.12a) or calreticulin (Figure 3.12b) levels were found between PANC-1 cells cultured with complete medium, pre-exercise or post-exercise conditioned serum.

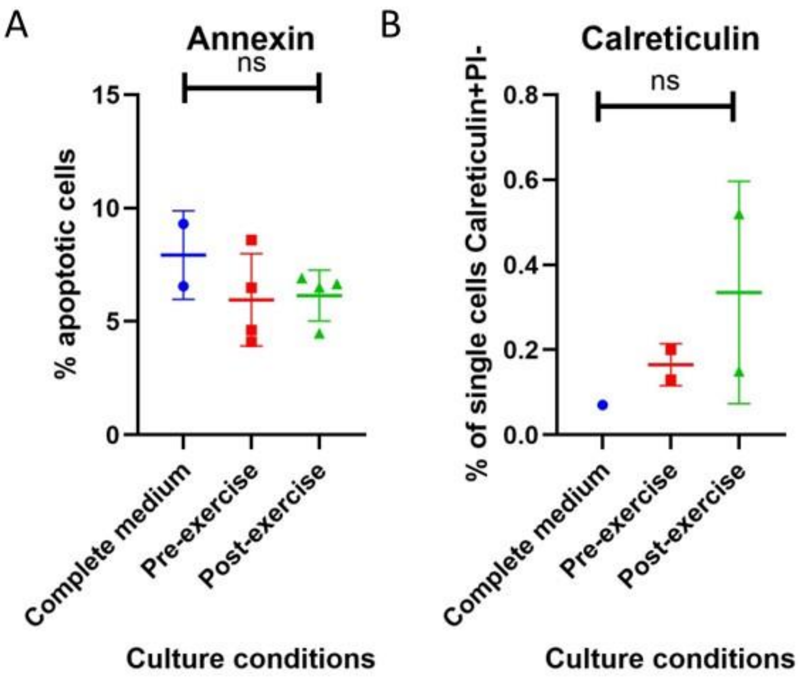


Figure 3.12: Annexin and Calreticulin were unchanged when exercise conditioned serum was cultured with PANC-1 cells

PANC-1 cells were cultured with either complete medium, 10% pre-exercise or 10% post-exercise conditioned serum for 24 hours (n=7). FITC-annexin V (BioLegend) and anti-calreticulin/Alexa-Fluor 647 (Novus) were added. After 15 minutes, following addition of binding buffer and centrifugation, incubation of samples took place on ice with propidium iodide for 15 minutes. The Cytek Aurora Flow Cytometry System and GraphPad prism 8.0.2 were used to analyse samples. Wilcoxon signed-rank tests were performed to ascertain significance. No statistically significant differences were seen in **A.** Annexin levels nor **B.** Calreticulin levels.

3.4.9: HSV1716 shows good infectivity of pancreatic cancer and mesothelioma cells

OVs are gaining interest as potential effective cancer treatments. OVs are a form of immunotherapy made to specifically target cancer cells in order to replicate, activate tumour lysis and promote cytotoxic immune responses against cancer (70). By drawing in effector immune populations via immunogenic cell death, OVs could work synergistically with exercise to produce ‘hotter’ tumour microenvironments and promote tumour cell death. Issues remain with safe and effective delivery of OVs to tumours, although T-VEC (genetically engineered from HSV-1) is approved for use in melanoma in the US and in the management of glioma in Japan (70).

The utility of HSV1716 in pancreatic cancer and mesothelioma was explored in this project as well as in combination with exercise-conditioned serum.

PANC-1 cell viability was similar across all different Multiplicity of Infection (MOI) of HSV1716 ranging from 0.3-10 (see Figure 3.13a). A similar trend was seen with MSTO-211H viability although high viability was seen in MOIs less than 1.25 (see Figure 3.13b). Therefore, an MOI of 1 was chosen to assess cell viability with exercise conditioned serum.

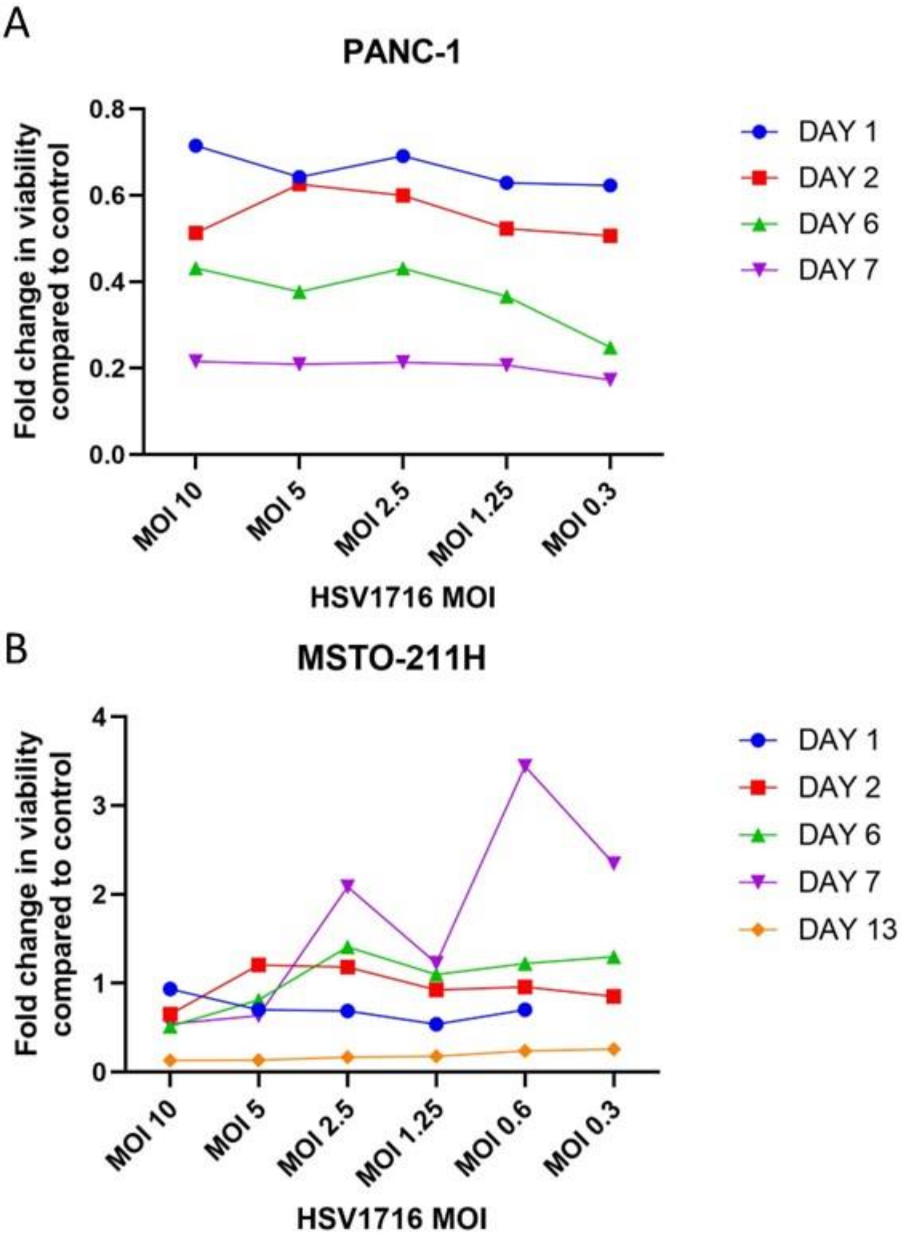


Figure 3.13: HSV1716 at multiplicity of infection (MOI) 1.25 is required to produce half-maximal inhibitory concentration (IC50) on PANC-1 and MSTO-211H cells

These figures demonstrate **A.** PANC-1 and **B.** MSTO-211H cell viability when inoculated with HSV1716 at MOI ranging from 0.3 - 10. 5 x 10^3 PANC-1 or MSTO-211H cells were seeded into wells on a 96-well plate in 100 µl of complete medium. HSV1716 was serially diluted in 50 µl of PBS to provide an MOI 10 (5 x 10^4 pfu), MOI 5 (2.5 x 10^4 pfu), MOI 2.5 (1.25 x 10^4 pfu), MOI 1.25 (6 x 10^3 pfu), MOI 0.6 (3 x 10^3 pfu) and MOI 0.2 (1.5 x 10^3 pfu). 50 µl of each viral concentration was added to separate wells and analysed in triplicate. Alamarblue© was added to each well on days 1, 2, 6, 7 and fluorescence was detected, following 4 hours incubation at 37°C, using the Ensight Microplate reader. Cells were incubated in complete medium alone were a positive control, whilst complete medium alone with no cells acted as a negative control. Cell viability was calculated as mean fluorescence at each MOI divided by mean fluorescence of the positive control wells.

Flow cytometry demonstrated 36.3% infectivity of PANC-1 cells with HSV1716 at 24 hours and 51.3% infectivity at 48 hours as demonstrated in Figures 3.14a-b and visualised with EVOS microscope in Figure 3.15C. Similarly, 35.8% infectivity of MSTO-211H cells with HSV1716 at 24 hours was seen and 59.3% infectivity at 48 hours (Figures 3.16-3.17).

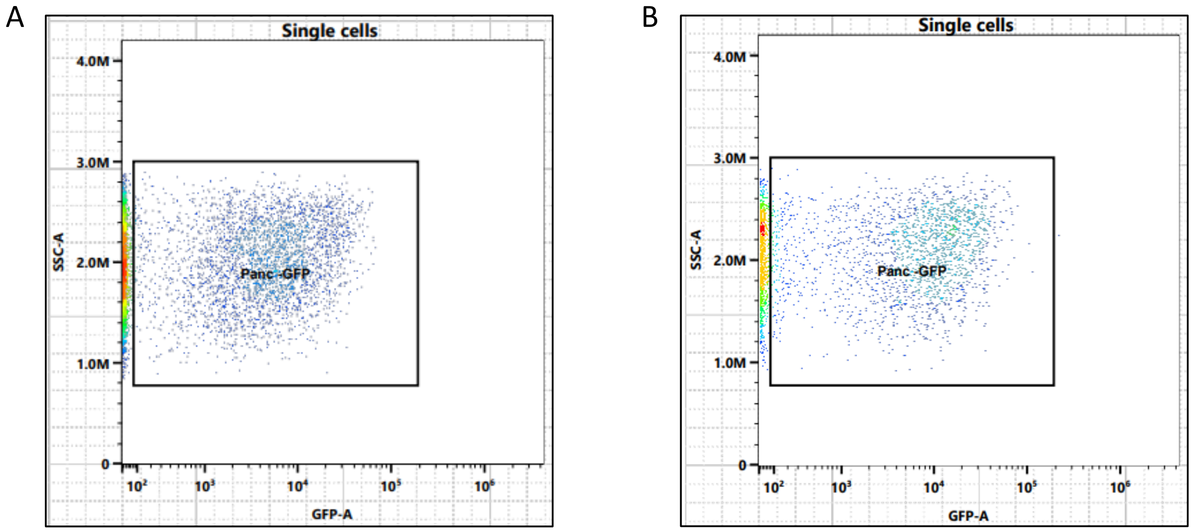


Figure 3.14: PANC-1 cell infectivity with GFP-expressing HSV1716

1 x 10^5 PANC-1 cells were seeded on a 6-well plate. Once confluent, cells were cultured with GFP-expressing HSV1716 at MOI 1. **A.** After 24 hours, cells were harvested and analysed using Cytek Aurora 3-laser Flow Cytometer. 36.3% of PANC-1 cells were shown to express GFP indicating HSV1716 infectivity. **B.** After 48 hours, flow cytometry was used to determine GFP-expressing cells. 51.3% of PANC-1 cells were shown to express GFP indicating HSV1716 infectivity.

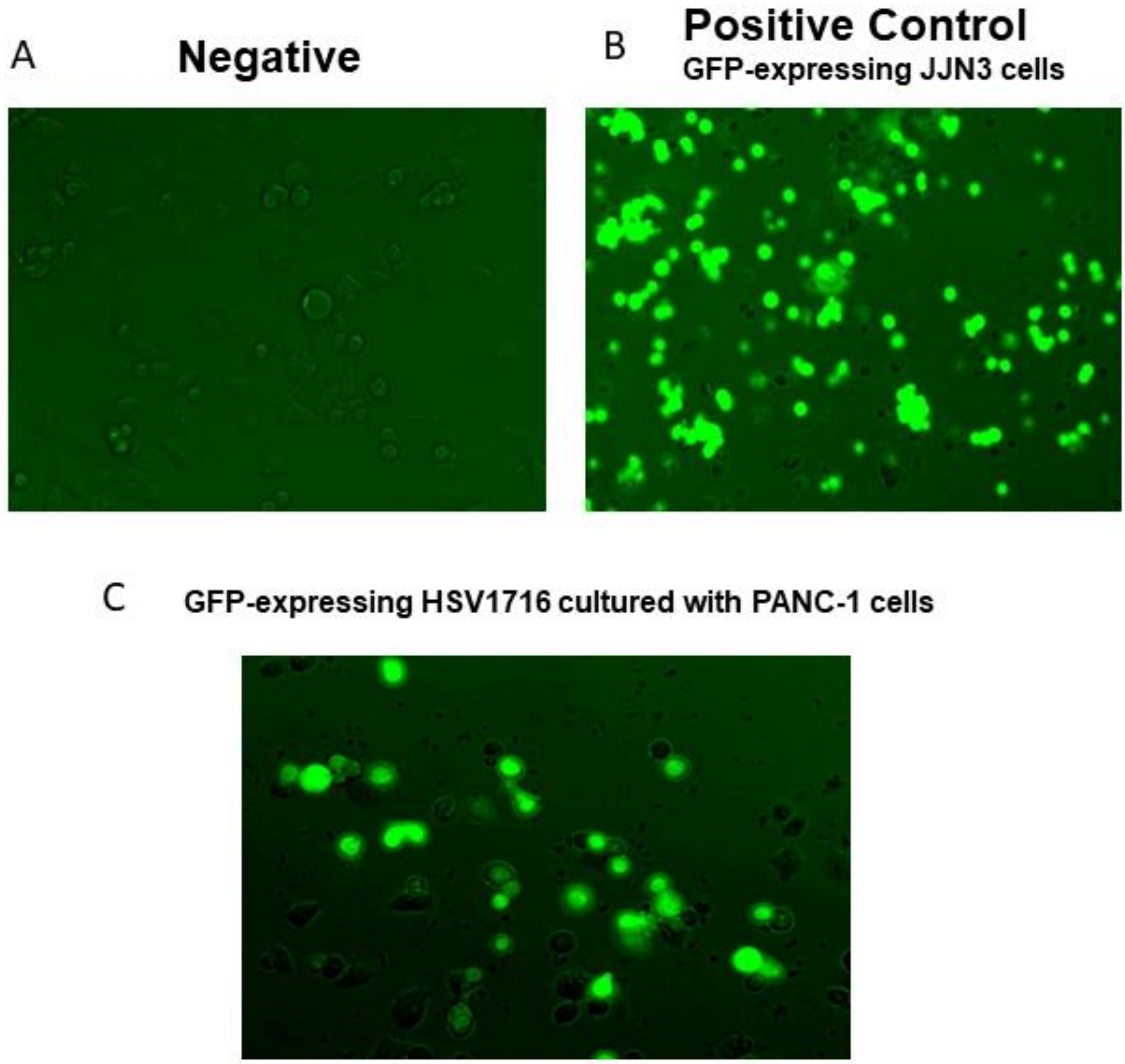


Figure 3.15: EVOS microscope used to demonstrate infectivity of PANC-1 cells with GFP expressing HSV1716

Representative images of n=3 experiments using the Invitrogen™ EVOS™ M7000 Imaging System (ThermoFisher Scientific) at 20X magnification. **A.** PANC-1 cells alone with no GFP tag (negative control). **B.** GFP-expressing JJN3 myeloma cells, demonstrating a positive control for GFP-labelled cells. **C.** GFP expressing HSV1716 co-cultured with PANC-1 cells. HSV1716 is able to enter and infect PANC-1 cells demonstrated by GFP fluorescence within the cells.

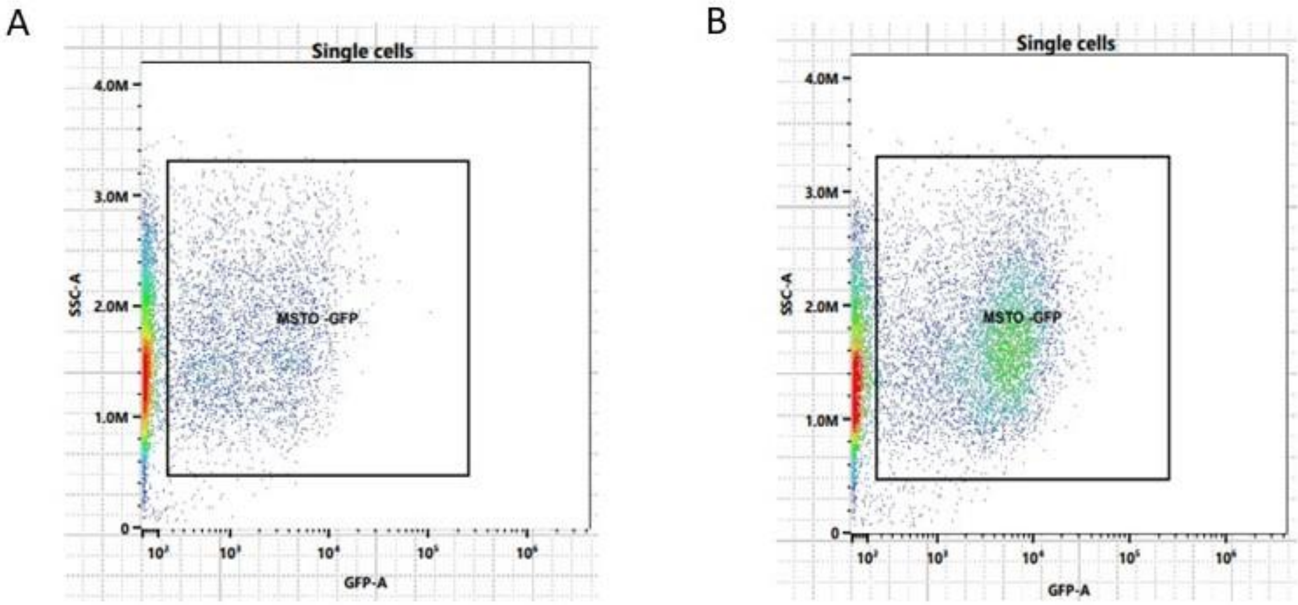


Figure 3.16: MSTO-211H cell infectivity with GFP-expressing HSV1716

1 x 10⁶ MSTO-211H cells were seeded on a 6-well plate. Once confluent, GFP-expressing HSV1716 at MOI 0.5 was added. **A.** After 24 hours, cells were harvested and analysed using Cytek Aurora 3-laser Flow Cytometer. 35.8% of MSTO-211H cells were shown to express GFP indicating HSV1716 infectivity. **B.** After 48 hours, flow cytometry was used to determine GFP-expressing cells. 59.3% of MSTO-211H cells were shown to express GFP indicating HSV1716 infectivity.

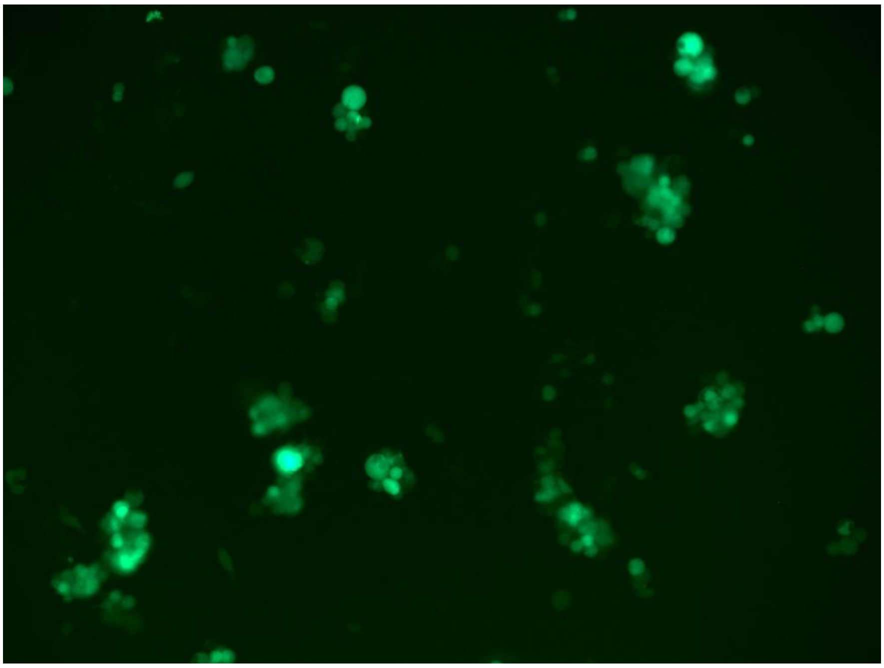


Figure 3.17: EVOS microscope used to demonstrate infectivity of MSTO-211H cells with GFP expressing HSV1716

Representative image of n=3 experiments using the Invitrogen™ EVOS™ M7000 Imaging System (ThermoFisher Scientific) at 20X magnification. Figure 3.17 illustrates that after co-culture with GFP expressing HSV1716 and MSTO cells, HSV1716 is able to enter and infect MSTO-211H demonstrated by GFP fluorescence within the cells.

3.4.10: Exercise conditioned serum and HSV1716 significantly reduces mesothelioma cell viability

Exercise-conditioned serum and HSV1716 significantly reduced MSTO-211H viability compared to pre-exercise serum and HSV1716 (p=0.0165) (Figures 3.18a-b). No significant differences in viability were seen with the same combinations when cultured with PANC-1 cells (Figure 3.18c).

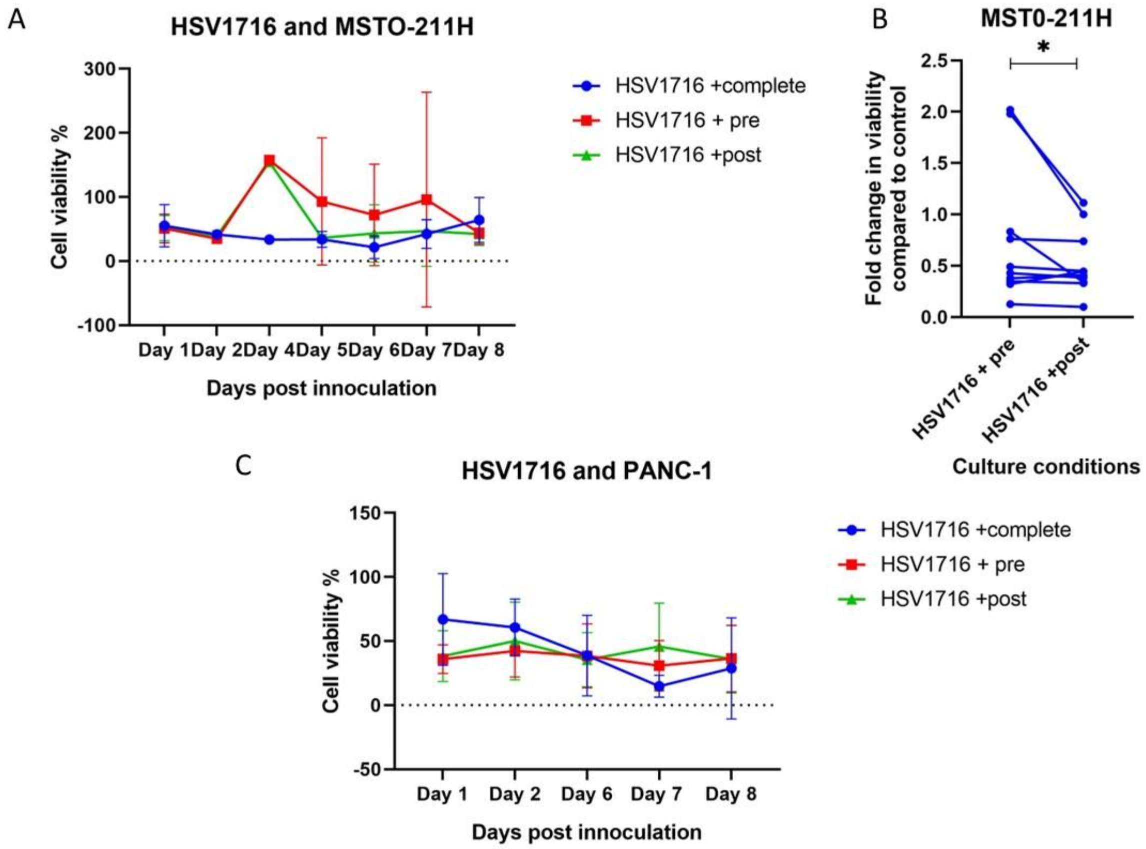


Figure 3.18: MSTO-211H and PANC-1 cell viability when cultured with different combinations of HSV1716 at multiplicity of infection (MOI) 1, pre- and post-exercise conditioned serum

A. MSTO-211H cell viability by the day and **B.** Overall viability from day 1-8 when inoculated with HSV1716 MOI 1. **C.** Demonstrates PANC-1 cell viability when inoculated with HSV1716 from days 1-8. 5 x 10^3 MSTO-211H or PANC-1 cells were seeded into wells on a 96-well plate in 100 µl of complete medium. After 24 hours incubation, medium was discarded, PBS was used to wash the wells and then cells were incubated with complete medium with HSV1716 MOI 1, pre-exercise conditioned serum with medium and HSV1716 MOI 1 or post-exercise conditioned serum with medium and HSV1716 MOI 1. Each combination was analysed in triplicate. Six participants pre- and post- exercised conditioned serum were explored. Alamarblue© was added to each well on days 1, 2, 6, 7 and 8 and fluorescence was detected, following 4 hours incubation at 37°C, using the Ensight Microplate reader. PANC-1 and MSTO-211H cells were incubated in complete medium alone and were a positive control, whilst complete medium alone with no cells acted as a negative control. Cell viability was calculated as mean fluorescence of selected wells divided by mean fluorescence of the positive control wells. Wilcoxon signed-rank tests were used to analyse the data within in GraphPad prism 8.0.2, where *$p<0.05$.

3.5: Discussion

Exercise has been shown to improve survival, quality of life and reduce cancer recurrence in different cancer settings (4,13). Growing evidence supports that aerobic exercise and resistance training promote anti-tumour immunity and may be pivotal in reprogramming cold tumour microenvironments to become hot. This study set out to determine the effect high intensity interval aerobic exercise had on systemic immunity in healthy volunteers. Furthermore, *in vitro* studies were performed to evaluate any exercise-induced changes within the participants' sera on cell viability, migration and apoptosis in mesothelioma and pancreatic cancer cells; tumours on different ends of the cold-hot spectrum.

3.5.1: Natural Killer (NK) cells

Our study has shown that a single bout of high intensity interval aerobic exercise in healthy volunteers stimulates a significant 32.6% increase in circulatory CD56 NK cells. There was an instantaneous systemic mobilisation of NK cells observed across all participants, irrespective of age, gender or baseline fitness. NK cells play a major role in innate immunity and help shape the adaptive immune response against cancer (22). Utilising this exercise-induced burst of NK cells may be of particular benefit to patients with metastatic cancer. Metastatic disease in all solid malignancies is reliant on the ability of cancer cells to evade regulated immune-mediated destruction during separation from the primary tumour, manipulation and restructuring of the extracellular matrix and movement through the circulatory system (71). CD8+ T cells, effector cells of adaptive immunity, rely on presentation of tumour antigen on MHC class I molecules, thus NK cells are able to act synergistically to eliminate cancer cells that lack MHC class I expression (72).

A high abundance of NK cells within the systemic circulation and intratumourally has been correlated with a favourable prognosis and specifically, a reduced metastatic potential in a range of hot to cold tumours including renal cell carcinoma (hot) and gastric cancer (cold) (71). Davis et al. correlated improved survival outcomes in patients with pancreatic cancer with higher circulatory NK cell count (73). Sottile et al. have shown that NK cell cytotoxicity is effective against mesothelioma cell lines and increased NK cell activity correlated to prolonged overall survival in mesothelioma patients (74). Our study findings support the literature regarding exercise-induced NK cell count and warrants further review in advanced mesothelioma and pancreatic cancer patients, which this project aims to achieve. Future work will include functional assays such as the NK Vue® Kit, using an enzyme-linked immunosorbent assay (ELISA) approach, which can measure NK cell activity via NK cell-specific IFNγ secretion (75). This would aim to demonstrate that the exercise-induced increase in NK cells translates to enhancement of NK cell function.

3.5.2: T regs

Complementing the increase in NK cells observed, we have demonstrated that a single bout of high intensity interval aerobic exercise in healthy volunteers reduced CD4+ T cells and notably CD4+CD25+ T cells by 28.5% and 31.0% respectively. CD4+ and CD25+ are markers found on Tregs, although not exclusively, which play an essential role in protecting against autoimmunity by dampening the immune response against self-antigens (76). However, they conversely advocate tumour propagation by promotion of immune tolerance to tumour-associated antigens (76,77). TMEs rich in Tregs potentiate a cold phenotype and correlate to immunotherapy resistance and poorer clinical outcomes (77). Treg-induced immune tolerance is achieved through multiple pathways including high expression of CTLA-4, an immune checkpoint that binds to CD80/CD86 on antigen presenting cells to provide inhibitory signals (78). Tregs are able to manipulate the cytokine profile by depleting IL-2, involved in T cell proliferation, as they highly express CD25 which consists of the IL-2 receptor α chain (79). They release IL-10, IL-35 and transforming growth factor beta (TGFβ) to create a hostile, ineffective environment for cytotoxic immune cells (79). Tregs are also directly cytotoxic to CD8+ T and NK cells via the release of perforin and granzyme B (78,79). Furthermore, they possess the chemokine receptors CXCR3, CCR4, CCR8 and CCR10 that allow intratumoural recruitment of Tregs potentiating the immunosuppressive tumour microenvironment (79).

Selective depletion of Tregs has been an area of interest to promote anti-tumour activity and potentiate anti-cancer treatment, such as ICIs. Initial studies had demonstrated Treg inhibition targeting CD25 exhibits favourable anti-tumour activity and promotion of CD8+ T cell intratumoural infiltration creating a hot microenvironment (77). However, CD25 is not specific to Tregs and its inhibition has been shown to dampen effector T cell activity as well (79).

Rech at al., demonstrated a significant decline in systemic Tregs in human leucocyte antigen-A2-positive (HLA-A2+) metastatic breast cancer patients receiving daclizumab, an anti-CD25 monoclonal antibody, in combination with a cancer vaccine targeting HLA-A2+ (80). A significant barrier to utilising and continuing ICI treatment includes the wide range of immune mediated toxicities which can be life threatening. This autoimmune phenomenon may be augmented by the removal of Tregs. However, this study defined two separate Treg populations CD45RA+ and CD45RA-, the latter implicated in tolerance to cancer proliferation and selectively inhibited by daclizumab, allowing CD45RA+ Tregs to carry out immune surveillance preventing auto-immunity (80). A reduction in immunosuppressive intratumoural Tregs whilst maintaining protection of healthy cells against auto-immunity is vital when designing a cancer intervention targeting Tregs to ensure safe and effective translation into clinical practice.

We have demonstrated that immediately post high intensity interval aerobic exercise, CD4+CD25+ Tregs decline. Tregs have been implicated in disease progression in both pancreatic cancer and mesothelioma (3,81), therefore our findings support a promising role of exercise in these patient groups. Exercise-induced Treg depletion could be utilised in cancer patients to promote anti-tumour activity, shift tumour microenvironments towards the hot end of the spectrum and potentiate ICI response.

There is conflicting evidence behind Tregs response to exercise, but this appears to be predicted by exercise duration and intensity. Wang et al. utilised mice models to demonstrate differing effects on Tregs between moderate and high intensity exercise (defined at 70% and 91% VO2 max respectively) with high intensity promoting Treg activity and moderate intensity exhibiting comparative Treg activity to controls (45). Of note, the exercise regime spanned over 6 weeks with increasing intensity and therefore, reflects exercise training rather than acute exercise explored in our study.

This may account for the differences seen and highlights the need to investigate the effect different exercise intensities have on Tregs in both murine models and humans with and without cancer.

Our findings show a trend towards greater exercise-induced reduction in CD4+CD25+ T cells in participants who regularly exercise at high intensity compared to moderate intensity, although this did not reach statistical significance, potentially due to the small subgroup sizes. No difference was observed in baseline Tregs between these two subgroups. Firm conclusions cannot be drawn due to the lack of statistical significance but warrants further review within a larger population.

Further subgroup analysis revealed participants under 35 years old had a greater reduction in CD4+ and CD4+CD25+ cells compared to those over 35 years old. This suggests there may be an inverse correlation between advancing age and exercise-induced immune responses. This would need to be reviewed when translating possible benefits in the advanced cancer patients who tend to be more of an older population.

Exercise has potential to optimise systemic immunity to promote anti-tumour cytotoxic activity with NK cells and reduce immune tolerance by Treg depletion. This exercise-induced shift towards a cytotoxic immune profile could support the actions of ICIs and enhance cancer response.

3.5.3: Monocytes

We found that the total number of CD14+ monocytes reduced by 17.9% immediately following acute high intensity exercise. Monocytes are another key player of the innate immune system involved in both anti-inflammatory (CD14+CD16-) and pro-inflammatory (CD14+CD16+) processes in order to maintain homeostasis (67). This heterogeneity makes it difficult to draw firm conclusions from our findings and studies are required to further clarify the changes within monocyte subtypes.

Circulatory monocytes are drawn by the hypoxic and chemokine-rich tumour microenvironment and are converted to TAMs (3). These can be classified as M1 (pro-inflammatory) and M2 (anti-inflammatory) subtypes, the latter contributing to tumour development and promotion of an immunosuppressive cold tumour microenvironment (82). Our study shows a reduction in CD14 monocytes with acute exercise. Further exploration is required to correlate an exercise-induced reduction in circulatory monocytes with a reduction of M2 intratumoural macrophages. This could present a further mechanism by which exercise-induced immune modulation could favour conversion of cold to hot tumours, by reducing the potential formation of tumour propagating TAMs.

3.5.4: Cell viability and cytokine bead arrays

In order to assess the effect exercise-conditioned serum could have on human mesothelioma and pancreatic cancer cell lines, cell viability assays using alamarBlue® were performed. To ensure the same participant serum-enriched medium was present during the incubation period, separate 24, 48 and 72-hour plates were seeded with each cell line. Cell viability was higher in both cell lines when cultured in their complete medium with 10% FBS comparative to human serum at most time points. No statistically significant difference in cell viability was seen between pre and post exercise-conditioned serum.

It is likely that the presence of anti-proliferative and pro-apoptotic factors within the serum of the human healthy volunteers leads to decreased cancer cell viability compared to complete medium with 10% FBS *in vitro*. IL-6, IL-7, IL-15 and myokines OSM, SPARC and irisin have all be shown to rise following aerobic exercise and/or resistance training and all demonstrate potential anti-cancer activity (17,25,28,29,31). Given the shift towards cytotoxicity seen in the leucocyte subpopulations with exercise, we used cytokine bead arrays to assess a small panel of both pro-inflammatory (IL-6, TNFα and IFNγ) and anti-inflammatory (IL-5 and IL-10) cytokines within the pre- and post- exercise conditioned serum of five participants. However, readings for both pre- and post- exercise conditioned serum were all comparable to the 0 pg/ml of standard indicating no cytokines were detected in any of the samples. This may be because the concentration of these cytokines were below the point of detection (10pg/ml).

Statistically significant changes in PANC-1 cell viability have been seen following incubation with serum taken from pancreatic cancer patients at baseline, 6 and 12 weeks into a whole body electromyostimulation (WB-EMS) programme; a method used to simulate the effect of resistance training (65). It would be important to address limitations within our study to further investigate exercise and cell viability in vitro.

Limitations include the use of healthy volunteers who all undertook regular exercise and therefore, cytokine changes seen with acute exercise may be modest compared to those who do not regularly exercise, the latter more accurately reflecting the advanced mesothelioma and pancreatic cancer population. Blood tests were done immediately following exercise but at no further time points. Studies have revealed that exercise-induced cytokine release, such as IL-6, can peak up to 2 hours after exercise completion (83). Therefore, there may not have been enough time to detect a significant change in cytokines accounting for the lack of cell viability difference between pre- and post-exercise conditioned serum. Further studies are required to look at different time points post exercise as well as to include patients with cancer. Previous pre-clinical studies have demonstrated anti-cancer properties with exercise training over weeks (28,29). Therefore, assessing cell viability with serum taken at different time points during repeated bouts of exercise over a longer duration of time needs to be explored, as well as using alternative methods such as proteomic panels.

3.5.5: Migration assays

Scratch assays demonstrated reduced pancreatic cancer cell migration with exercise-conditioned serum compared to matched pre-exercise serum. Reduced cell migration was observed when cells were incubated with complete medium compared to human serum, indicating the presence of proliferative factors within human serum supporting migration of cancer cells. However, exercise appears to be able to skew this towards an anti-migratory profile.

Evidence suggests that exercise-conditioned serum would be enriched with myokines, such as irisin (32,65). Recombinant irisin has been shown to exert anti-migratory properties on pancreatic cancer cells by disrupting the epithelial-mesenchymal transition, preventing detachment from surrounding cells by increasing E-cadherin and reducing motility by diminishing vimentin (32). Schwappacher et al. provided evidence supporting anti-migratory properties of WB-EMS against pancreatic cancer cells implicating other myokines such as IL-10 and CCL4 (65).

Scratch assays were not successful with MSTO-211H cells despite many troubleshooting steps including optimising the cessation of proliferation with different concentrations of mitomycin C and different durations of serum-free media exposure. MSTO-211H cells were also seeded on collagen-coated well plates but this did not prevent cell detachment during this step and on initiation of the scratch. Therefore, transwell migration assays were used demonstrating a reduced migration with exercise-conditioned serum.

This study demonstrated the ability of physical aerobic exercise to inhibit pancreatic cancer cell and mesothelioma migration, which could translate to the reduction of the metastatic potential of these cancers in vivo. In turn, this could significantly enhance survival outcomes.

3.5.6: Apoptosis

Caspase 3/7 assays demonstrated a clear increase in apoptotic activity of PANC-1 and MSTO-211H cells when cultured with post-exercise conditioned serum. Loss of protective apoptotic pathways occur early within the development of these cancers and it is partly due to this persistent evasion of apoptosis which renders anti-cancer treatments relatively futile (84). Exercise-mediated apoptosis of tumour cells would be invaluable in improving pancreatic cancer and mesothelioma treatments.

3.5.7: HSV1716

HSV1716 utility as a virotherapy in mesothelioma has been reviewed. Danson et al (85) demonstrated safety of intrapleural administration of HSV1716 in mesothelioma patients and demonstrated that HSV1716 was able to elicit a favourable cytotoxic cytokine profile. This is particularly promising as evidence for enhancing immunotherapy given the modest benefits seen with dual ICIs (nivolumab and ipilumumab) in advanced/ unresectable mesothelioma. As previously discussed, mesothelioma TMEs contain a mixture of effector and immune tolerant cells which are likely to inhibit the full potential of immunotherapy(10). HSV1716 may provide the trigger to the immune system needed to reap the full benefits of ICIs. Furthermore, we have demonstrated that a combination of HSV1716 and post-exercise conditioned serum led to decreased mesothelioma cell viability in vitro. Further patient studies are warranted to explore the combination of OV/ICIs in combination with exercise to enhance treatment response and improve survival outcomes.

3.6: Conclusion

In summary, our findings have shown a single bout of high intensity interval aerobic exercise significantly increases circulatory NK cells and reduces Tregs providing the optimum environment to boost anti-cancer activity. This provides a strong basis to investigate whether this effect can be seen systemically and intratumourally in patients with advanced cancer. When cultured with exercise-conditioned serum, mesothelioma and pancreatic cancer cell apoptosis is significantly increased, and migration is reduced. A combination of exercise/ HSV1716 demonstrated significant reduction in mesothelioma viability highlighting a potential effective treatment combination between exercise and immunotherapy. Further work is required to assess the mechanisms behind these anti-cancer effects and whether these can be accentuated by exercise training in cancer patients.

Chapter 4
In vivo effects of exercise on pancreatic cancer and mesothelioma

4.1: Introduction

Orthotopic mouse models allow the accurate representation of TMEs incorporating factors such as tumour vasculature, hypoxia, stroma and immunity in the correct anatomical position, demonstrating superiority to subcutaneous tumour models in allowing translatability to clinical studies (86). Therefore, we have chosen orthotopic models to examine the detailed interactions between exercise physiology, an ICI (anti-PD1) and systemic and intratumoural immunity in pancreatic cancer and mesothelioma.

Kurz et al (25) demonstrated the feasibility of C57BL/6 mice running on a treadmill the day following orthotopic surgical implantation of pancreatic cancer cells into the pancreata. We have developed a similar model using the Panc02 cell line rather than KPC. This is key to allow the full benefit of exercise alongside immunotherapy on tumour growth, metastases and TME modulation in pancreatic cancer which follows an aggressive clinical path with rapid deterioration. Our collaborators, Digifico et al (87) developed the intraperitoneal mesothelioma mouse model used in this chapter. The utility of exercise in established mesothelioma models has not been explored to the best of our knowledge, and this research sets out to describe the exercise-induced effects in conjunction with an immune checkpoint inhibitor within this established mouse mesothelioma model.

Mouse studies involving aerobic exercise usually utilise either voluntary wheel running or involuntary treadmill running. Treadmill running allows standardisation of activity across all mice, providing a more accurate evaluation of the effects of aerobic exercise. Moderate intensity exercise has been utilised in many exercise-oncology mouse studies, demonstrating favourable anti-cancer immune responses from increased intratumoural NK and CD8 T cells and reduced MDSCs (25,42,88). However, moderate intensity encompasses a range of activity from 30-60 minutes/day for 5/days a week at a speed of 9-15 metres/minute. Given the advanced age and probable frailty of the target populations with advanced pancreatic cancer and mesothelioma, exercise at a further reduced level was chosen to allow better translatability into clinical practice. In order to maximise the potential immune benefit from exercise and capitalise on the specific release of myokines, resistance training was incorporated into the exercise protocol. Irisin and SPARC are myokines shown to be significantly elevated following resistance training and have exerted suppressive properties against different cancers including pancreatic, prostate and breast cancers (29,31,32).

A recent study demonstrated significant improvements in overall survival and myeloma bone disease in combination with standard chemotherapy treatment with both low and moderate-intensity exercise in a mouse myeloma model over chemotherapy alone (66). Similarly to our healthy volunteer study, they demonstrated systemic Treg reduction and NK cell increase within the exercising mice groups. Upregulation of IL-15, a key myokine determining NK cell activation, has been described in different mouse exercise models exhibiting anti-cancer effects including in pancreatic cancer (25,66,89). Kurz et al (25) show that the combination of treadmill running and anti-PD1 leads to reduced tumour weight in a pancreatic cancer mouse model. In this chapter, we aim to expand on the treatment response to assess primary tumour necrosis and the effect on metastatic disease.

The poor prognosis seen in pancreatic cancer is largely because of its nature to rapidly invade and metastasise, with liver metastases being the most common and lethal (90). Epithelial-mesenchymal transition (EMT) underpins the mechanism of tumour cell detachment, intravascular and distant tissue invasion which is dependent on multiple factors including matrix metalloproteinases 2 and 9 (MMP2 /9), E-cadherin, vimentin and transforming growth factor-β1 (91). Yazdani et al (92) demonstrated a significant reduction in liver metastases with exercise training within a colorectal cancer mouse model. Supporting this, Ge et al (91) showed within a lung cancer mouse model, moderate and high intensity reduced MMP9 and MMP2 levels respectively. Inhibiting the metastatic potential would be vital in improving prognosis across all cancers because when tumour cells migrate and invade distant organs, an initially curable cancer may become incurable, drastically reducing life expectancy.

This chapter aims to explore whether these benefits can be applied to pancreatic cancer and mesothelioma. Although mesothelioma does not commonly distantly metastasise, lung and liver are possible sites and therefore will be assessed for metastatic disease (88).

Macrophages can be found in abundance within the TMEs of pancreatic cancer and mesothelioma (93,94). TAMs display complex phenotypes lying on a spectrum from pro-inflammatory (M1) to anti-inflammatory (M2), the latter more predominant in these cold tumours promoting tumour growth and immune evasion (93). Morrisson et al demonstrated that a graded moderate-intensity treadmill running programme polarised TAMs towards an M1 phenotype with upregulated TNFα expression indicating activation in an ovarian cancer mouse model (95). As part of this PhD project, I supervised Phoebe Southam, a student on the Sheffield Undergraduate Research Experience (SURE) scheme, which provides opportunities for enthusiastic students to be actively involved in research of their interest. We examined the effect of exercise and/or PD1 on M1 and M2 macrophages within the mouse mesothelioma tumours using immunofluorescent staining.

In this chapter, we build on the exercise-induced effects on systemic immunity and cancer cells in vitro from the last chapter and examine the combination of aerobic and resistance exercise with an ICI on the primary tumour, TME and distant disease in pancreatic cancer and mesothelioma in vivo.

4.2: Hypothesis

1) Exercise combined with anti-PD1 therapy will modulate the TME in pancreatic cancer and mesothelioma mouse models to favour anti-cancer immunity (increased CD8 T cells and NK cells and reduced MDSCs and Tregs)
2) Exercise combined with anti-PD1 will enhance treatment response by increasing tumour necrosis and reducing metastatic disease

4.3: Aims and Objectives

1) Assess the feasibility of treadmill running and resistance training in orthotopic pancreatic cancer and mesothelioma mouse models
2) Assess the impact of exercise and exercise/anti-PD1 therapy on pancreatic cancer and mesothelioma TME in mouse models
3) Assess the effect of exercise and exercise/anti-PD1 therapy on tumour necrosis and metastatic disease

4.4: Results

4.4.1: Pancreatic cancer mouse studies

4.4.1.1: Exercise is feasible in a surgical orthotopic pancreatic cancer mouse model

A pilot study was performed assessing the feasibility of treadmill running, horizontal screen resistance training (where mice hung upside down on a wired screen) and a combination of both in 12 C57BL/6 mice who had pancreatic cancer cells surgically implanted into their pancreata. Mice were able to tolerate both treadmill running and horizontal screen training from day 2 following surgery and throughout the study.

Although growth of the pancreatic tumour was possible to track in certain mice using IVIS, not all tumours were visible. At the end of the study, mice were humanely killed, and all mice had developed tumours, which were then harvested for flow cytometry and histological analysis.

Figure 4.1 demonstrates the 12 C57BL/6 mice in the pilot pancreatic cancer study from weeks 1-4. By week 4, 7 out of the 12 mice had visible bioluminescent signalling from tumours with IVIS. However, all mice had tumours on dissection suggesting the IVIS was unable to detect these. All mice maintained weight and good condition throughout the study.

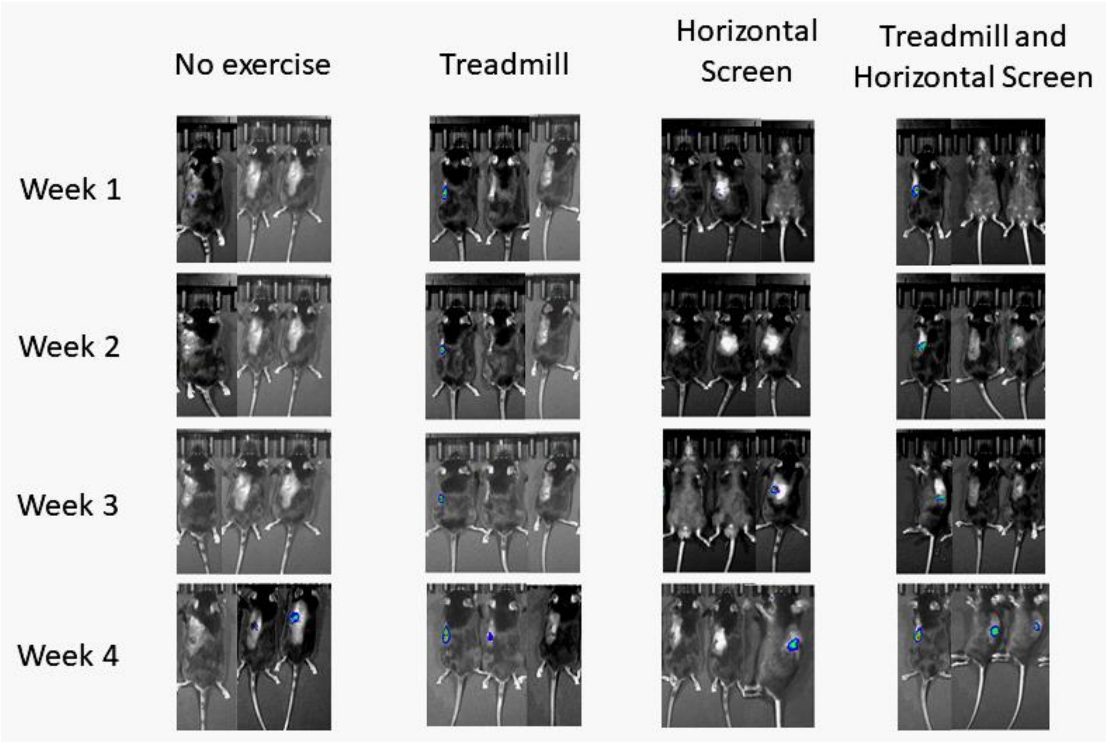


Figure 4.1: IVIS images of mice from pilot study

C57Bl/6 mice underwent orthotopic pancreatic cancer implantation with 4×10^5 luciferase-labelled Panc02 cells. Mice were categorised into four groups with n=3/group: no exercise, treadmill only 4 days/week, horizontal screen resistance training 2 days/week and a combination of treadmill and horizontal screen training. IVIS was used to assess bioluminescence of the tumour weekly illustrated. 100µl of 6mg/ml of D-luciferin was injected intraperitoneally 7 minutes prior to imaging. Mice were positioned in an induction chamber and anaesthetised with 5% isoflurane before being moved to the IVIS chamber where they remained on 2% isoflurane as maintenance during imaging.

4.4.1.2: Treadmill running and resistance training causes a trend towards reduced intratumoural Tregs and raised NK cells in pancreatic cancer

Figures 4.2A-H demonstrate the proportions of leucocyte subpopulations present within the TMEs of mice in the different groups of the pilot study. Although not significant, a trend towards a reduction of CD4+CD25+ Tregs and an increase in NK cells within the tumour microenvironment were seen in the exercising groups (p=0.1000 and p=0.3727 respectively). These trends were seen in mice that underwent treadmill running and/ or horizontal screen resistance training. This complements the systemic immune response findings from the healthy volunteer study and highlights the potential key role resistance training could bring alongside aerobic exercise.

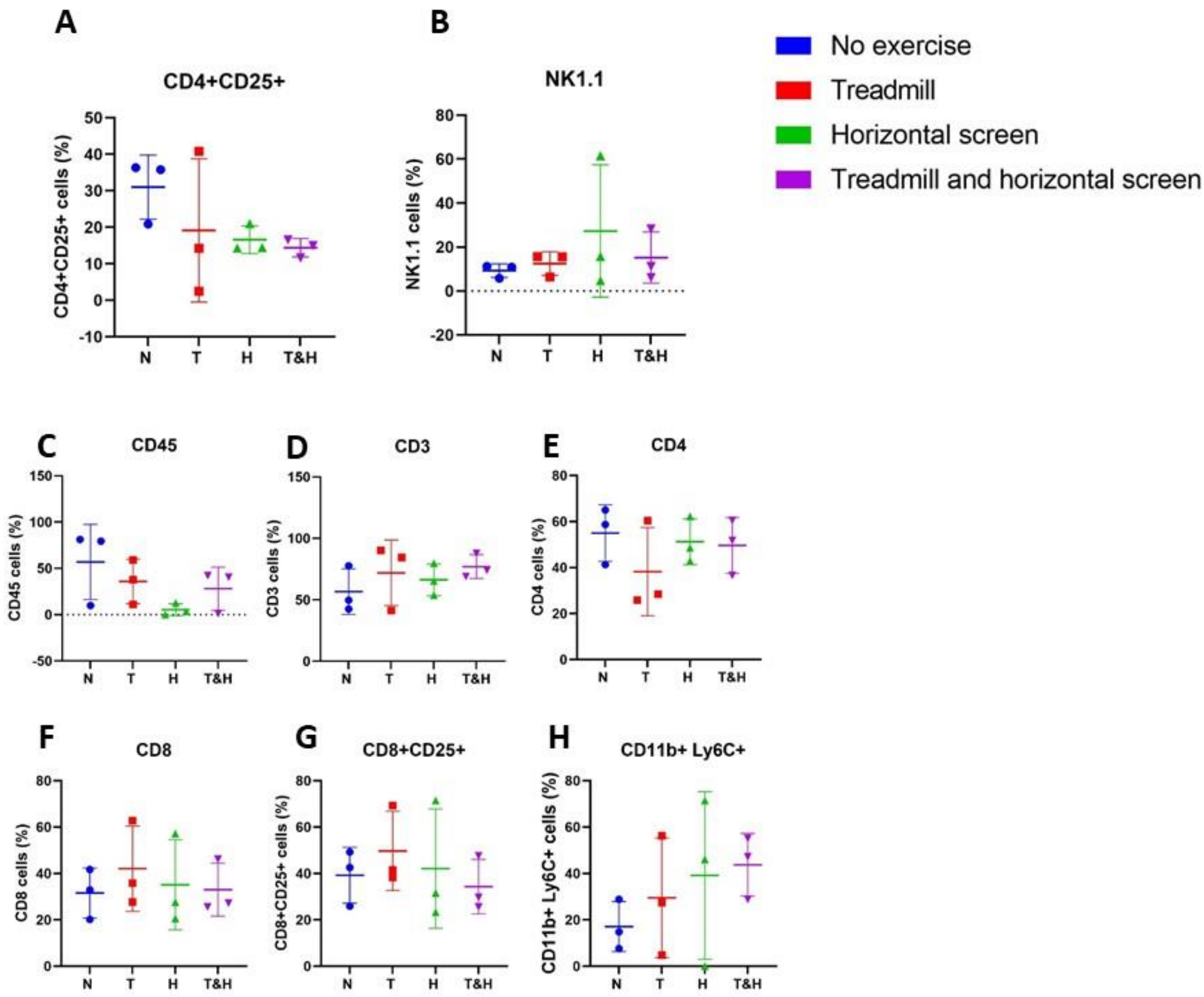


Figure 4.2: Aerobic exercise and resistance training causes a trend towards favourable anti-cancer tumour immune microenvironment

On completion of the study, pancreatic tumours were dissected, dissociated to single-cell solutions and stained with the following antibodies: **A.** CD45, **B.** CD3, **C.** CD3+CD4+, **D.** CD3+CD4+CD25+, **E.** CD3+CD8+, **F.** CD3+CD8+CD25+, **G.** NK1.1 and **H.** CD11b+Ly6C+. Samples were run through the Cytek ® Aurora Flow Cytometry system and SpectroFlo ® software was used for analysis. Trends towards reduced CD4+CD25+ Tregs and increase in activated CD8+CD25+ cytotoxic T cells were seen with treadmill and horizontal screen training. A trend towards raised NK1.1 cells was seen with horizontal screen and treadmill training. One-way ANOVA and Mann-Whitney tests were used to ascertain statistical significance, which was not reached.

4.4.1.3: Exercise reduces liver micrometastases in pancreatic cancer

Figures 4.3A-C demonstrate the significant reduction in metastatic liver disease burden in the mice that underwent exercise, specifically treadmill running. Significant differences were demonstrated between cohorts with regards to the number and size of liver micrometastases (p=0.0382 and p=0.0179 respectively). A 92.9% reduction in the size of liver micrometastases (p=0.0087) was observed in the treadmill running mice (Figure 4.3c). Large metastases were not noted within the liver although micrometastases were common as demonstrated in Figure 4.4.

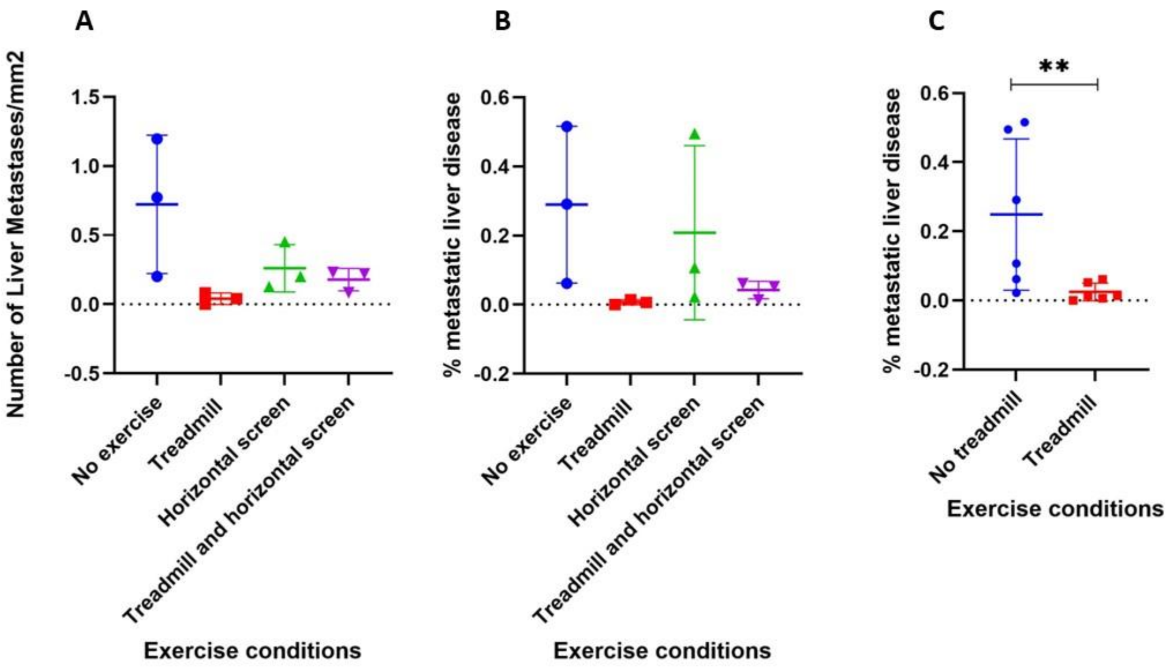


Figure 4.3: Aerobic exercise reduces the number and burden of liver metastases

At the end of the study, livers were collected from all mice and kept in 4% paraformaldehyde for 72 hours. Livers were then placed in cassettes in 70% ethanol, sectioned and H&E stained. H&E slides were scanned using the Epredia Panoramic 250 Flash III Slide Scanner up to 40X magnification. One liver lobe from each mouse was then analysed using SlideViewer, areas of metastatic disease were counted and measured comparatively to the area of the liver lobe to calculate the number of liver metastases and the percentage of liver affected by metastatic disease. Kruskal-Wallis test was used as a one-way ANOVA to assess statistical difference between more than 2 groups. Mann-Whitney tests were used to ascertain statistical significance between two groups. A statistically significant difference in the **A.** Number and **B.** Area of liver metastases was seen between groups **C.** Comparing all mice who underwent treadmill running versus no running, a significant reduction in metastatic area was seen in the treadmill cohorts ** $p<0.005$, * $p<0.05$.

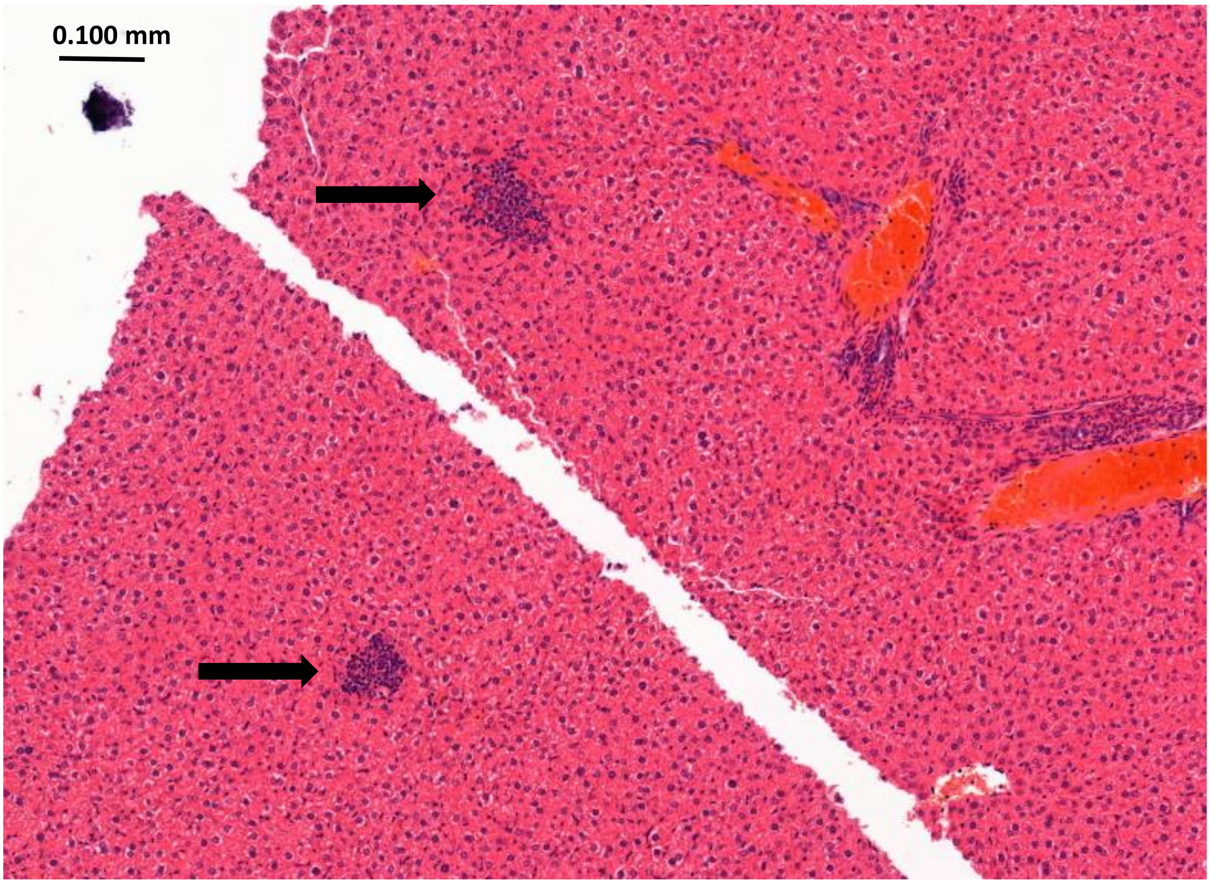


Figure 4.4: Micrometastatic liver disease on haematoxylin and eosin (H&E) slides

The black arrows point to the micrometastases present on liver histology. The number and area of liver micrometastases per mm² of liver were measured using SlideViewer™ at 10X magnification.

4.4.1.4: Exercise/ anti-PD1 therapy combination was feasible in this orthotopic pancreatic cancer mouse model

A study with 20 C57BL/6 mice was performed using the same orthotopic pancreatic cancer model. Mice were separated into 4 groups: no exercise/ isotype drug, no exercise/ anti-PD1 therapy, exercise/ isotype drug and exercise/ anti-PD1 therapy. Due to surgical complications at the start of the study, 1 mouse from each of the isotype drug groups required humane killing prior to data collection. Not all mice demonstrated IVIS visible tumours, although on dissection at the end of study, all mice had developed tumours. Figure 4.5a demonstrates the bioluminescent signalling of the mice from different groups, highlighting the difficulty in tracking tumours throughout the study. Figure 4.5b illustrates the mean weight of mice in each group throughout the study, with no mice reaching the humane endpoint of 15% weight loss.

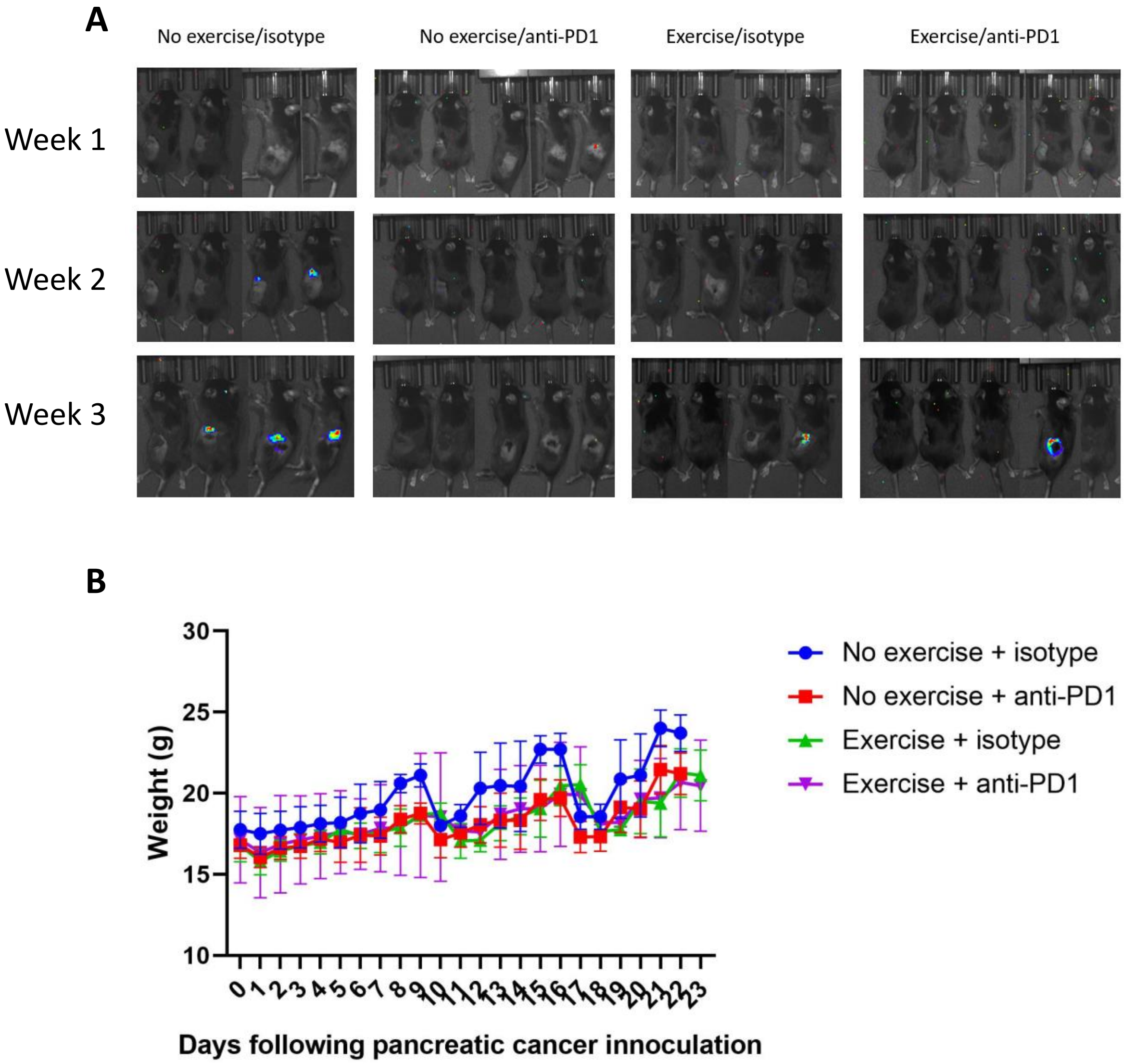


Figure 4.5: IVIS images and weights of mice from pancreatic cancer/anti-PD1 study

C57Bl/6 mice underwent orthotopic pancreatic cancer implantation with $4x10^5$ luciferase-labelled Panc02 cells. Mice were categorised into four groups with n=5/group: no exercise + isotype or anti-PD1 and exercise + isotype or anti-PD1. **A.** IVIS was used to assess bioluminescence of the tumour weekly. **B.** Mice were weighed daily and monitored for weight loss with a humane endpoint of 15% weight loss.

4.4.1.5: Exercise reduces immune tolerance within pancreatic tumours

TME analysis with flow cytometry demonstrated a significant reduction in MDSCs (73.5%, p=0.0426, Figure 4.6a) and Tregs (89.0%, p=0.0115, Figure 4.6b) in the exercising groups. Figures 4.7a-h demonstrate the percentage of different leucocyte populations within the mouse groups. Figure 4.7d illustrates a trend towards the lowest intratumoural Tregs in the exercise/anti-PD1 group. Interestingly, drops in

CD8 cytotoxic T cells (Figure 4.7e) and NK cells (Figure 4.7g) were seen in the exercise/anti-PD1 group comparative to the no exercise/anti-PD1group (p=0.0286 for both). The relevance of this is discussed later when these immune populations were quantified using immunofluorescent staining. No significance was found in other leucocyte populations.

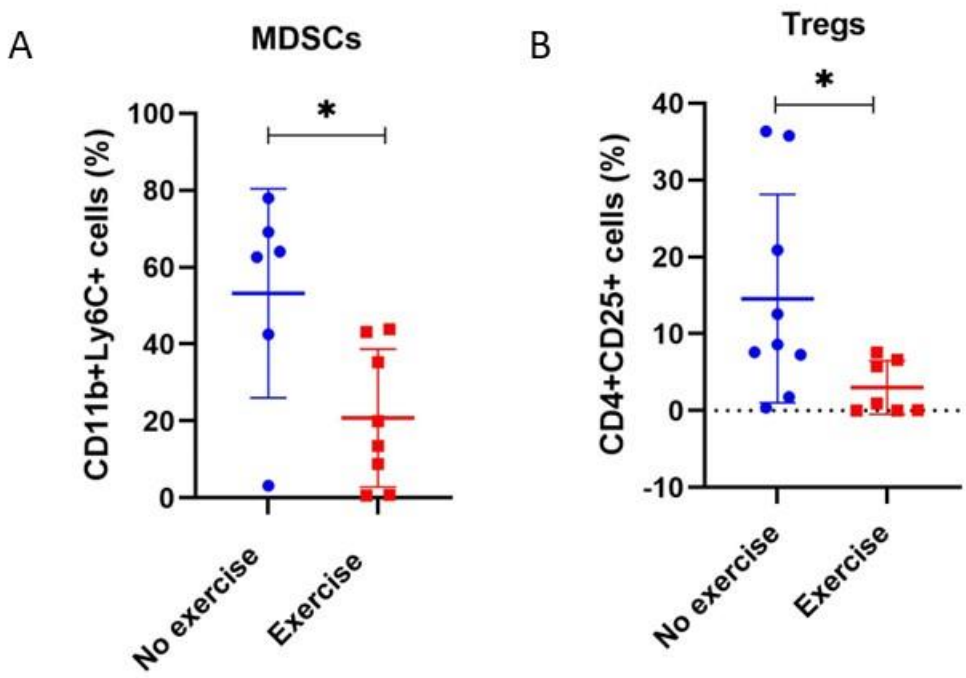


Figure 4.6: Aerobic exercise and resistance training causes a reduction in MDSCs and Tregs within the pancreatic TME

Flow cytometry was used to assess an immune panel including CD45, CD11b, Lyc6c, CD3, CD4 and CD25 on the dispersed pancreatic tumours on completion of the study. **A.** A 73.5% reduction in Ly6c+CD11b+ MDSCs (p=0.0426) and **B.** An 89.0% reduction in CD4+CD25+ Tregs (p=0.0115) were seen with exercise + both anti-PD1 and isotype. Mann-Whitney tests were used to ascertain statistical significance where *p<0.05

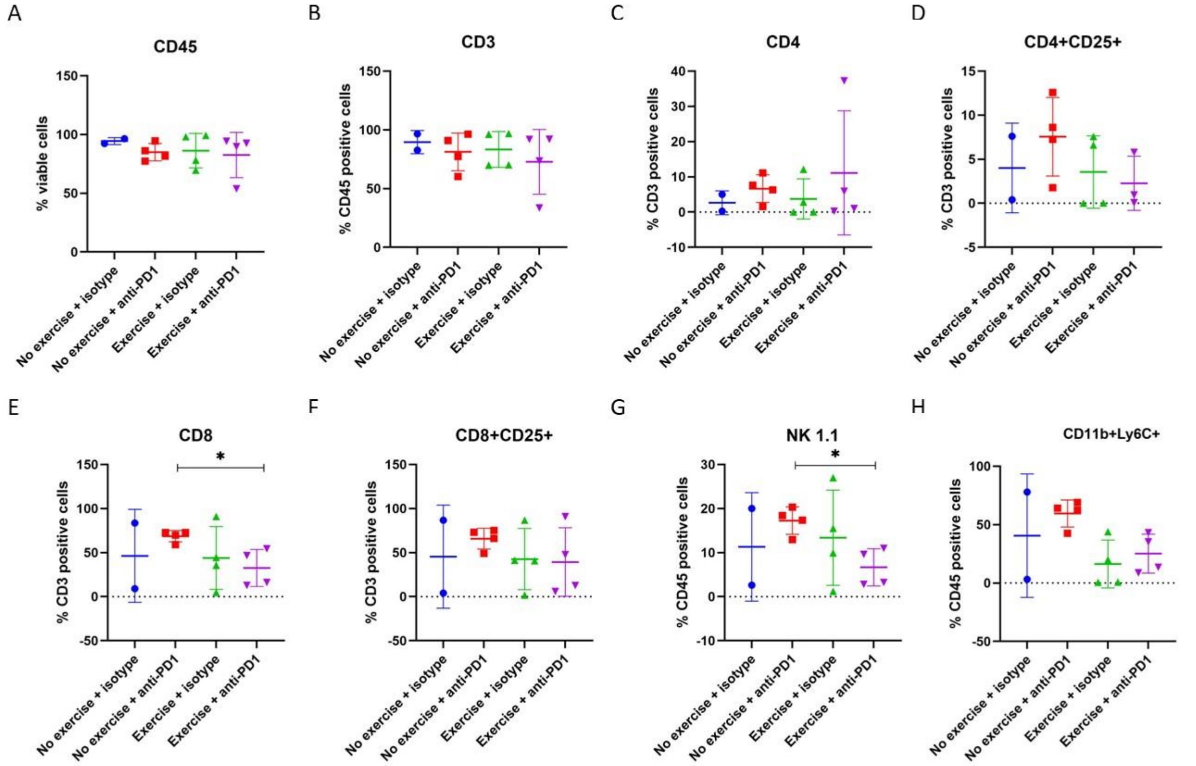
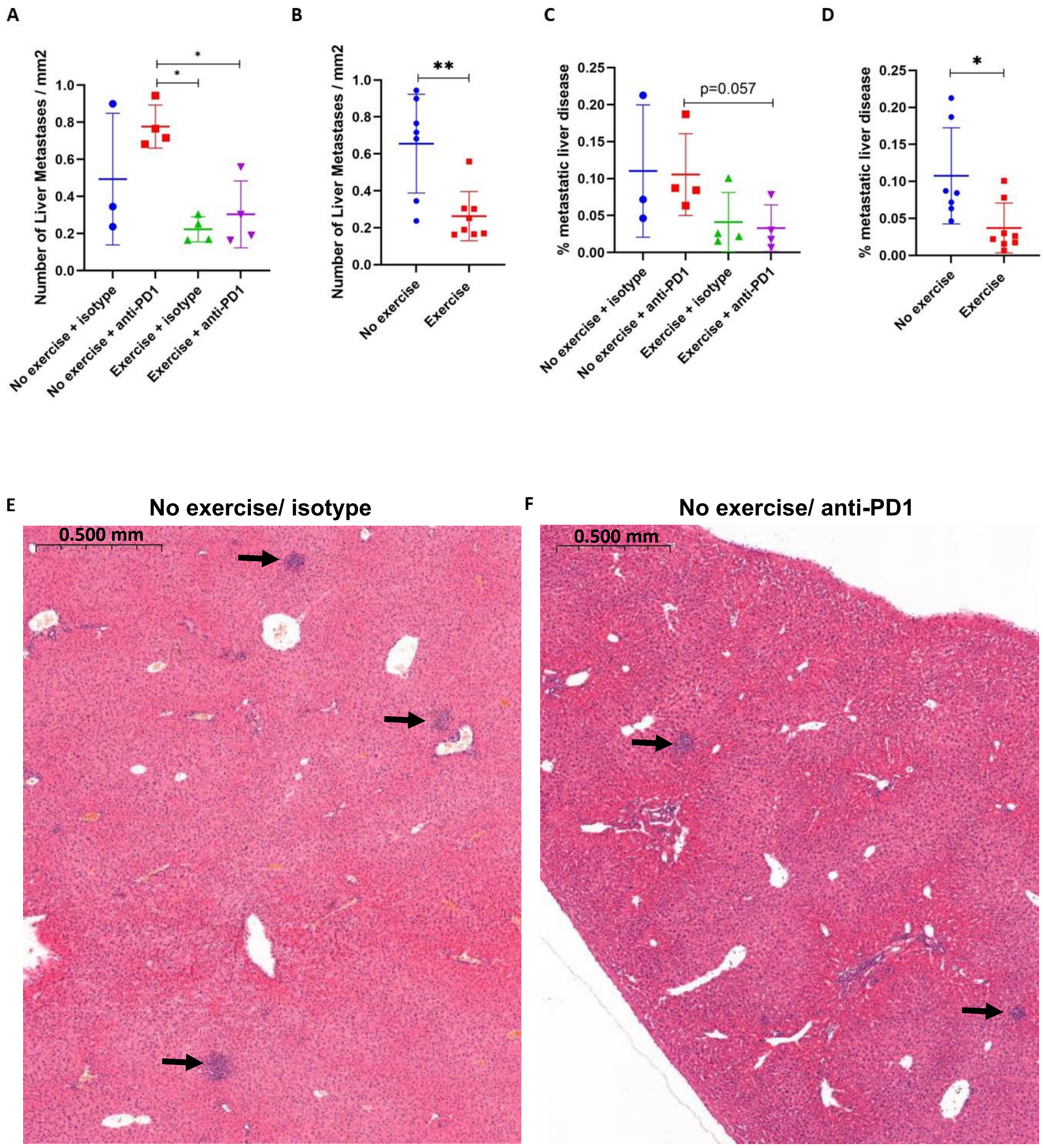


Figure 4.7: Exercise and anti-PD1 induced modulations within the pancreatic TME

A full flow cytometry immune panel assessed in the pancreatic tumours on completion of the study included: **A.** CD45, **B.** CD3, **C.** CD3+CD4+, **D.** CD3+CD4+CD25+, **E.** CD3+CD8+, **F.** CD3+CD8+CD25+, **G.** NK1.1 and **H.** CD11b+Ly6C+. Trends towards reduced CD11b+Ly6C+ MDSCs and CD4+CD25+ Tregs were seen in the exercise groups, although not significant. A reduction in CD8 T and NK cells was seen in the exercise/anti-PD1 groups comparative to the non-exercise/anti-PD1 groups. One-way ANOVA and Mann-Whitney tests were used to ascertain statistical significance where *p<0.05

4.4.1.6: Exercise reduces liver micrometastases in pancreatic cancer irrespective of drug treatment

Similarly to the pilot study, significant reductions were seen in the number and size of liver micrometastases within the exercising groups (p=0.0059 and p=0.02 respectively). Figure 4.8a demonstrates significant reductions in the number of liver micrometastases in both exercise/ isotype and exercise/ anti-PD1 groups comparative to the no exercise/anti-PD1 group (p=0.0286 for both). Figure 4.8b highlights this significance is strengthened when analysing exercising cohorts together comparative to non-exercising groups (p=0.0059). To expand on the nature of these micrometastases, the area of metastatic disease was measured comparative to liver size. Although significance was not reached, a trend towards smaller micrometastases was seen in the exercise/ anti-PD1 group, as seen in Figure 4.8c (p=0.057). This size reduction did reach significance when exercising cohorts were compared to non-exercising (p=0.0205, Figure 4.8d). Amongst the mice that underwent exercise, there was no statistically significant difference seen between isotype or anti-PD1 treatment. Figure 4.9 illustrates the differences in the number and size of liver micrometastases between the different groups on H&E slides.



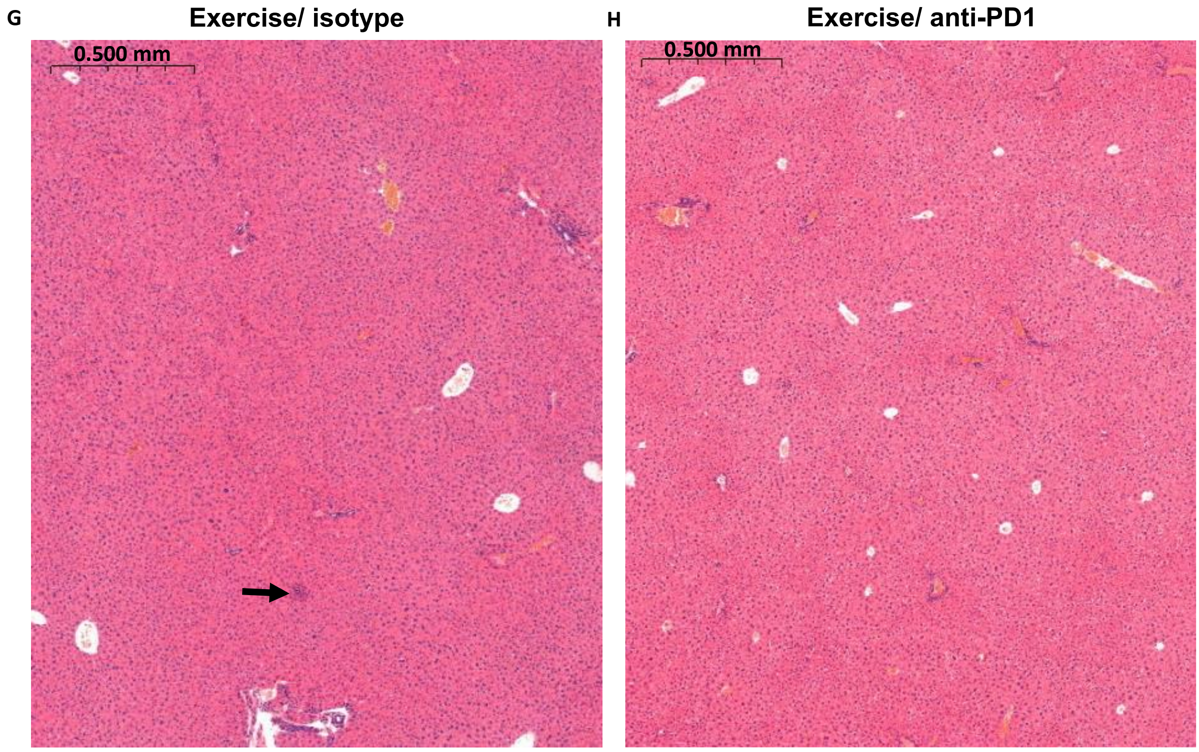


Figure 4.8: Exercise reduces the number and burden of liver micrometastases

At the end of the study, livers were collected from all mice and kept in 4% paraformaldehyde for 48 hours. Livers were then placed in cassettes in 70% ethanol, sectioned and H&E stained. H&E slides were scanned using the Epredia Panoramic 250 Flash III Slide Scanner up to 40X magnification. One liver lobe from each mouse was then analysed using SlideViewer, areas of micrometastatic disease were counted and measured comparatively to the area of the liver lobe to calculate **A-B.** The number of liver micrometastases and **C-D.** The percentage of liver affected by micrometastatic disease. H&Es slides illustrating the differences in the number and size of liver micrometastases seen in mice within the different groups: **E.** No exercise/ isotype **F.** No exercise/ anti-PD1 **G.** Exercise/ isotype **H.** Exercise/ anti-PD1. Black arrows point to the micrometastases. Mann-Whitney tests were used to ascertain statistical significance. ** p<0.005, * p<0.05

4.4.1.7: Exercise and anti-PD1 therapy significantly enhances tumour response

In order to characterise treatment response, the degree of tumour necrosis was measured using SlideViewer with scanned H&E slides, visualised in Figure 4.9. The larger the area of necrosis (non-viable tumour tissue), the greater the effect of treatment on the primary tumour. Figure 4.10a shows the significant difference in tumour necrosis seen between groups (p=0.0177) with the largest degree of necrosis seen in tumours of the exercise/anti-PD1 group comparative to the no exercise/ isotype group (p=0.0269) and the no exercise/ anti-PD1 group (p=0.0244). Although a clear trend is demonstrated indicating increased tumour necrosis in the exercise/anti-PD1 group compared to the exercise/isotype group, this did not reach significance (p=0.1968). Figure 4.10b demonstrates the significant impact exercise exerts on tumour necrosis irrespective of drug treatment (p=0.0087).

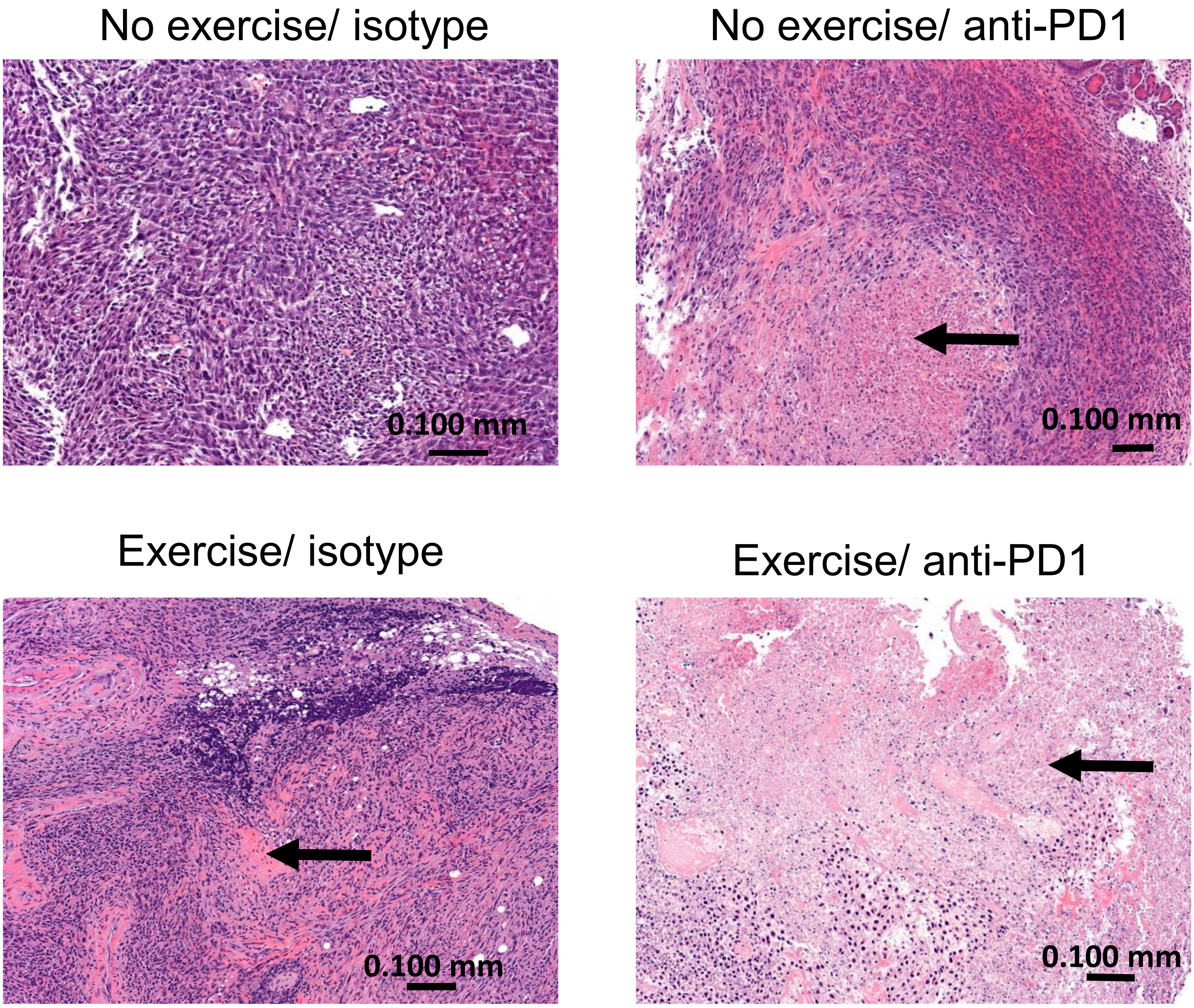


Figure 4.9: H&E slides of pancreatic tumours from pancreatic cancer mouse study

Figure 4.9 demonstrates the H&E slides of tumours from the pancreatic cancer mouse study. Following end of study, pancreatic tumours were harvested and placed in 4% paraformaldehyde for 24 hours. Tumours were then put into 70% ethanol, paraffin wax embedded, sectioned and H&E stained. The Epredia Panoramic 250 Flash III Slide Scanner was used to scan the images and SlideViewer was used to measure the percentage of necrosis (pale pink appearance with loss of nuclei indicated by black arrows) compared to total tumour area.

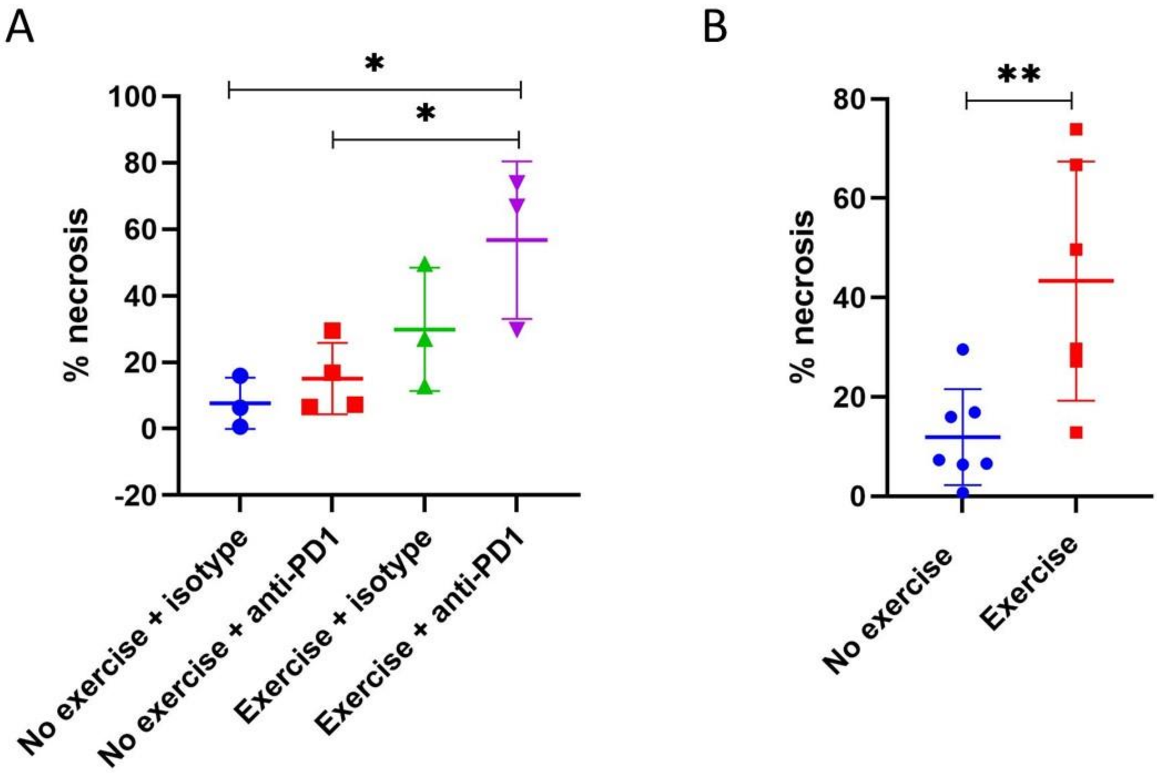


Figure 4.10: Exercise/anti-PD1 combination significantly increases tumour necrosis

Areas of necrosis were measured relative to whole tumour size from H&E slides using SlideViewer. Shapiro-Wilk tests confirmed normality of the data and ordinary one-way ANOVA and unpaired T-tests were used to ascertain differences in necrotic areas between tumours from different groups. **A.** A significant difference was seen in tumour necrosis between the different groups, p=0.0177. Exercise/ anti-PD1 significantly increased the degree to tumour necrosis comparative to the no exercise /isotype and the no exercise/anti-PD1 group **B.** Increased tumour necrosis was seen in the exercise cohorts, where *p<0.05, **P<0.01

4.4.1.8: Exercise and anti-PD1 therapy significantly increases intratumoural CD8 T cell infiltration and CD8+PD1+ T cell expression

To further explore the immune climate of these tumours, immunofluorescent staining with CD8 and PD1 was performed. Immunofluorescent staining of pancreatic tumours demonstrated a significant difference in CD8+ cytotoxic T cell infiltration, with the highest concentration found in the exercise/anti-PD1 group (p=0.0368), specifically noted around areas of necrosis, which have previously been demonstrated to be significantly raised in this group. Figure 4.11 demonstrates the disparity in cytotoxic T cell infiltration within the tumours of the different groups.

To complement this, PD1 expression was assessed on T cells to assess activation. Small numbers of T cells were evident in the pancreatic tumour histology from the no exercise/PD1 group. There were few T cells present in the pancreatic tumours of the no exercise/ isotype group and most did not show signs of activation due to the lack of PD1 expression. Conversely, there were more T cells present in the exercise/PD1 group all of which expressed PD1 as demonstrated in Figure 4.12. This indicates that the combination of exercise/anti-pd1 not only increases cytotoxic T cell infiltration intratumourally but also enhances PD1 expression on these T cells. Figures 4.13 illustrates the ‘cold’ immune climate of pancreatic tumours with low levels of CD8 T cells and PD1 expression in the non-exercising groups and exercise/isotype group and the transformation towards a ‘hotter’ climate in the exercise/anti-PD1 group with raised CD8+PD1+ T cell counts (p=0.0247).

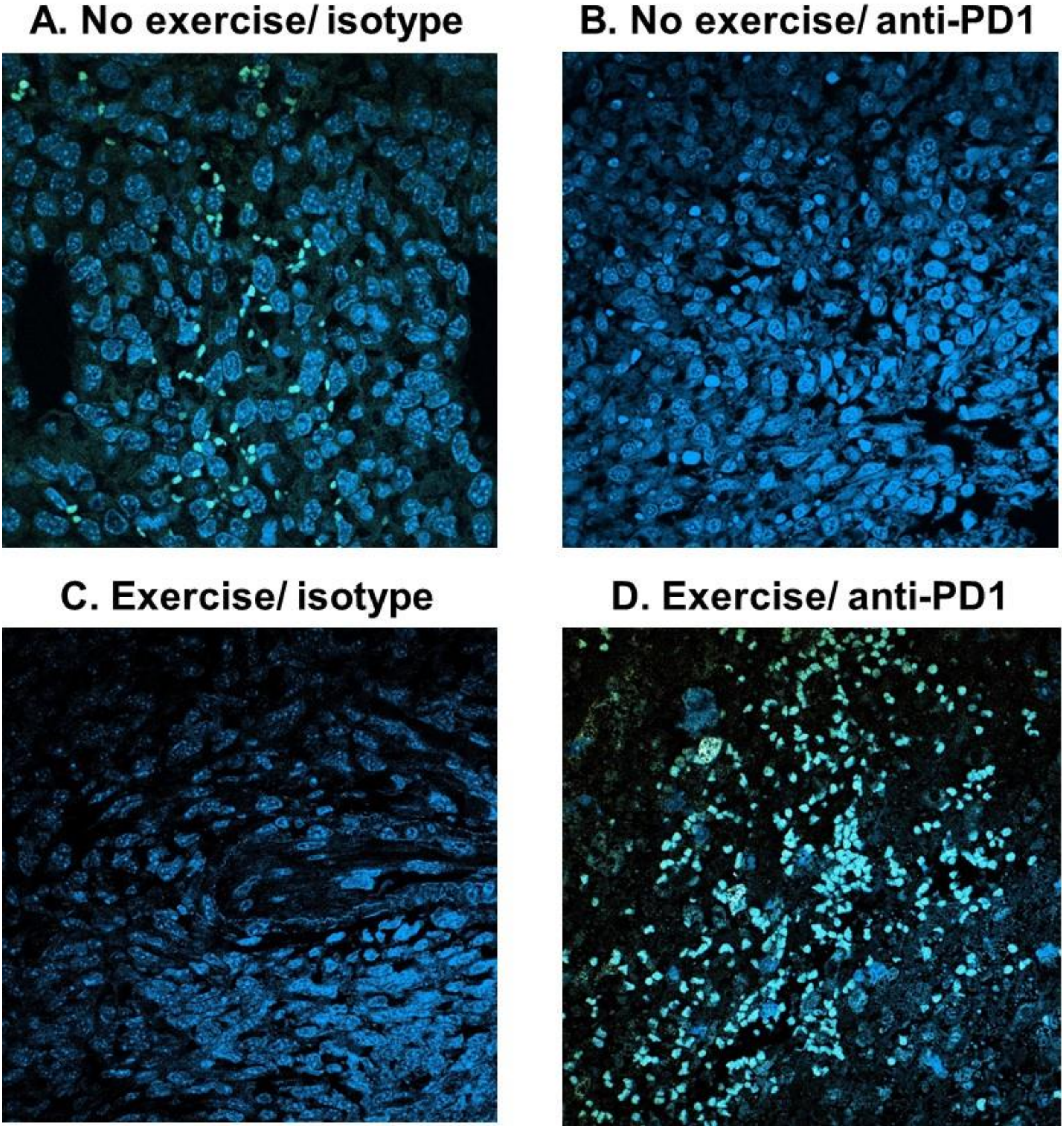


Figure 4.11: CD8 T cell infiltration is significantly increased with exercise training and anti-PD1 therapy

Representative images demonstrate the immunofluorescent stained tumours from the pancreatic cancer mouse study from **A.** the no exercise/ isotype group, **B.** the no exercise / anti-PD1 group, **C.** exercise/ isotype group and **D.** the exercise/ anti-PD1 group. Paraffin wax-embedded slides were dewaxed in xylene and hydrated sequentially with 100%, 95%, 90% and 70% ethanol. Following antigen retrieval with a heater, slides were stained with CD8-FITC (green) antibodies along with DAPI (blue). Zeiss LSM 980 Airyscan confocal was used to visualise slides at 40X magnification.

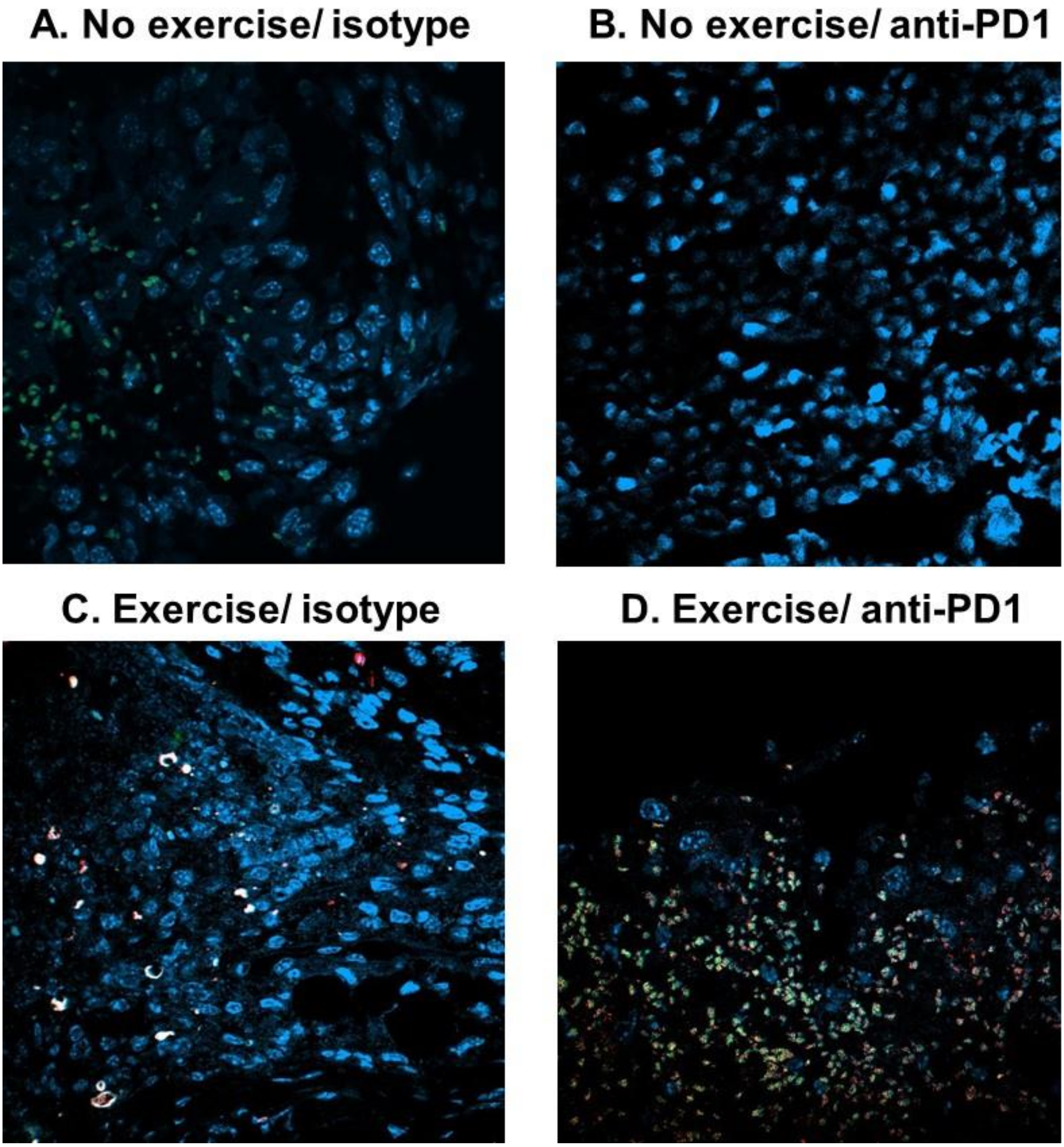


Figure 6: Activated CD8+PD1+ T cells are significantly upregulated with exercise training and anti-PD1 therapy

Representative images demonstrate the immunofluorescent stained tumours from the pancreatic cancer mouse study from **A.** the no exercise/ isotype group, **B.** the no exercise / anti-PD1 group, **C.** exercise/ isotype group and **D.** the exercise/ anti-PD1 group. Paraffin wax-embedded slides were dewaxed in xylene and hydrated sequentially with 100%, 95%, 90% and 70% ethanol. Following antigen retrieval with a heater, slides were stained with CD8-FITC (green) and PD1-PE (red) antibodies along with DAPI (blue). Zeiss LSM 980 Airyscan confocal was used to visualise slides at 40X magnification.

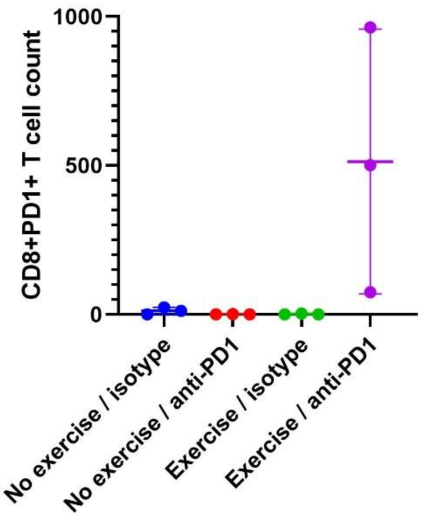


Figure 4.13: Exercise/anti-PD1 combination significantly increases cytotoxic T cell infiltration and PD1 expression in the tumour

Following staining with CD8 and PD1 antibodies, slides were images with the Zeiss LSM 980 Airyscan confocal and Fiji ImageJ was used to count CD8+PD1+ T cells within the tumours of different mice groups. Kruskal-Wallis non-parametric one-way ANOVA ascertained statistically significant differences between the median of the groups for CD8+PD1+ T cells (p=0.0247).

4.4.1.9: Exercise was not shown to affect NK cell levels within the pancreatic TME

Given we observed significant increases in systemic NK cells in exercising healthy volunteers, we aimed to explore the NK cell populations within these tumours using immunofluorescent staining. Limited NK cell infiltration was demonstrated in tumours from each group (1 tumour from each group was reviewed), with the only NK cell presence noted within the exercise/isotype group as demonstrated in Figure 4.14.

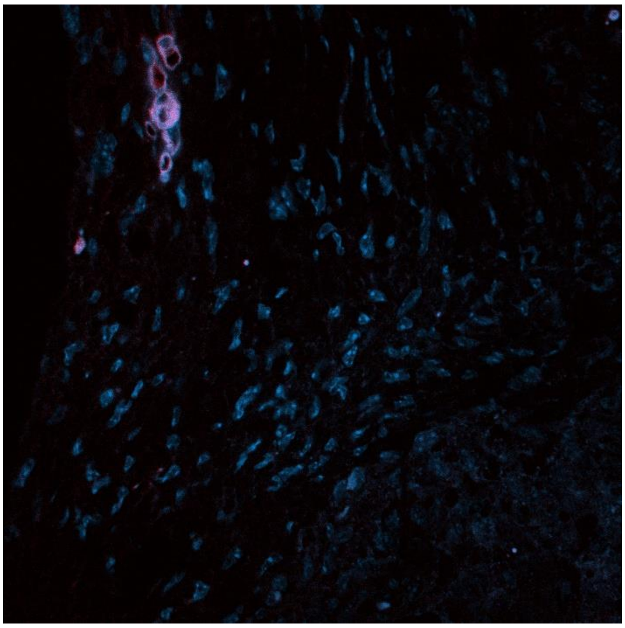


Figure 4.14: NK cells within the pancreatic tumour of a mouse in the exercise/ isotype group

Representative immunofluorescent staining of a tumour from the pancreatic cancer mouse study (exercise/ isotype group). Paraffin wax-embedded slides were dewaxed in xylene and hydrated sequentially with 100%, 95%, 90% and 70% ethanol. Following antigen retrieval with a heater, slides were stained with NK1.1-PE (red) along with DAPI (blue). Zeiss LSM 980 Airyscan confocal was used to visualise slides at 40X magnification.

4.4.2: Mesothelioma mouse study

4.4.2.1: A modified aerobic exercise and resistance training regime was feasible in an orthotopic mesothelioma mouse model

Figure 4.15 illustrates the IVIS imaging of the 24 BALB/c mice from weeks 1-3 of the study who underwent intraperitoneal injection of mesothelioma cells. Unlike the previous studies, strong bioluminescent intensities were detected in all mice corresponding to the presence of mesothelioma tumours in all mice. Mice were able to exercise from day 2 following intraperitoneal injection of AB-1 mesothelioma cells. However, mice were not able to complete 15 minutes of treadmill running despite modifications including interval running and reduced speed from 12 metres/min to 9 metres/min. Therefore, modified time of 10 minutes of treadmill running was carried out across all mice at speed 12 metres/min. Mice were able to perform the horizontal screen exercise.

4.4.2.2: Exercise causes a trend towards reduced radiance in mesothelioma tumours

Figure 4.16a demonstrates a trend towards increased bioluminescent radiance detected from tumours of the no exercise/anti-PD1 compared to the exercise/anti-PD1 group (p=0.0706) and lower radiances detected from the exercising cohorts (Figure 4.16b), although not statistically significant (p=0.25).

All mice slowly gained or maintained their weight throughout the study with no significant differences seen between groups (see Figure 4.16c). One mouse in the no exercise/anti-PD1 group had to be killed one day early on Day 15 due to reaching the humane endpoint of poor body condition (reduced mobility and mouse grimace score). All other mice were culled on Day 16, with only one of the mice (in the no exercise/isotype group) reaching the humane endpoint of poor body condition. In general, all mice within the exercise groups maintained good body condition and mobility.

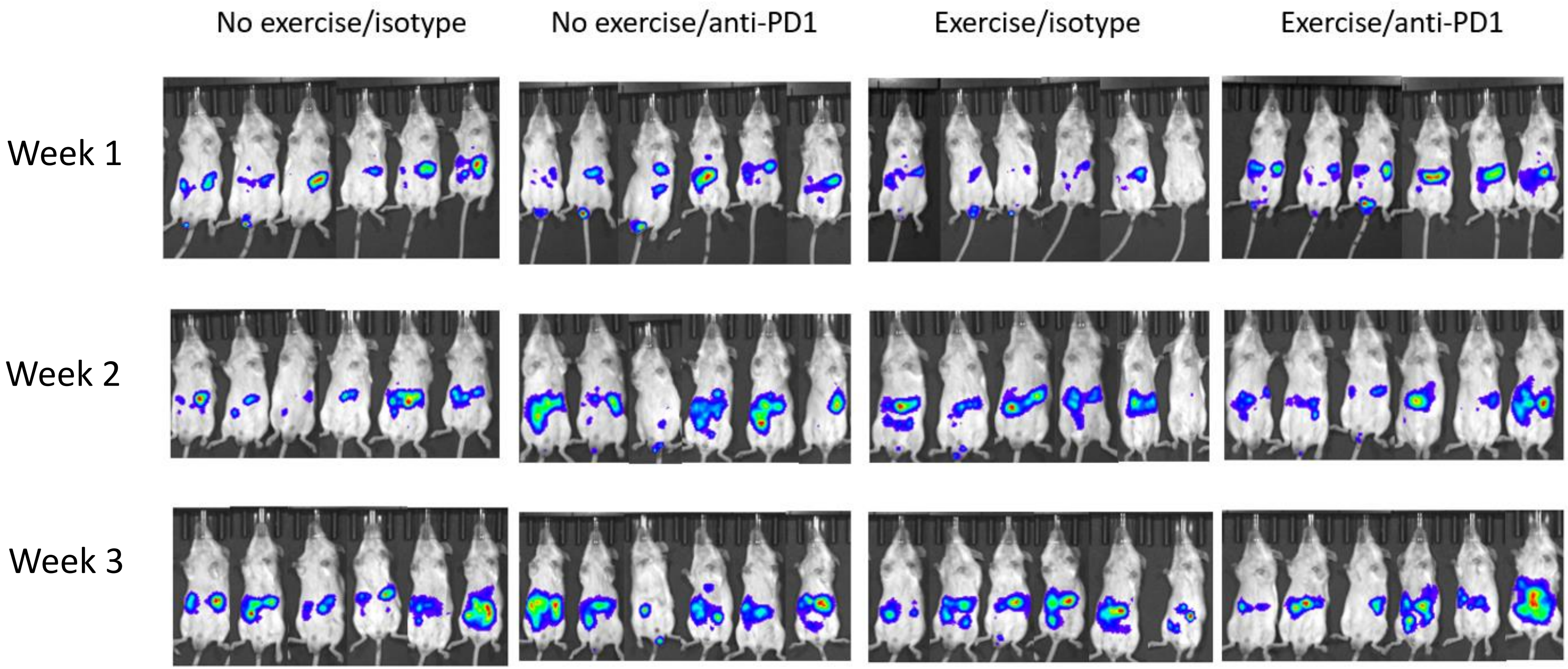


Figure 4.15: IVIS images of mice from mesothelioma study

BALB/c mice underwent intraperitoneal mesothelioma implantation with 1×10^5 luciferase-labelled AB-1 cells. Mice were categorised into four groups: exercise with anti-PD1, exercise with isotype, no exercise with anti-PD1 and no exercise with isotype. In Vivo Imaging Systems (IVIS) was used to assess bioluminescence of the tumour. 100µl of 6mg/ml of D-luciferin was injected intraperitoneally 7 minutes prior to imaging. Mice were positioned in an induction chamber and anaesthetised with 4% isoflurane before being moved to the IVIS chamber where they remained on 2% isoflurane as maintenance during imaging.

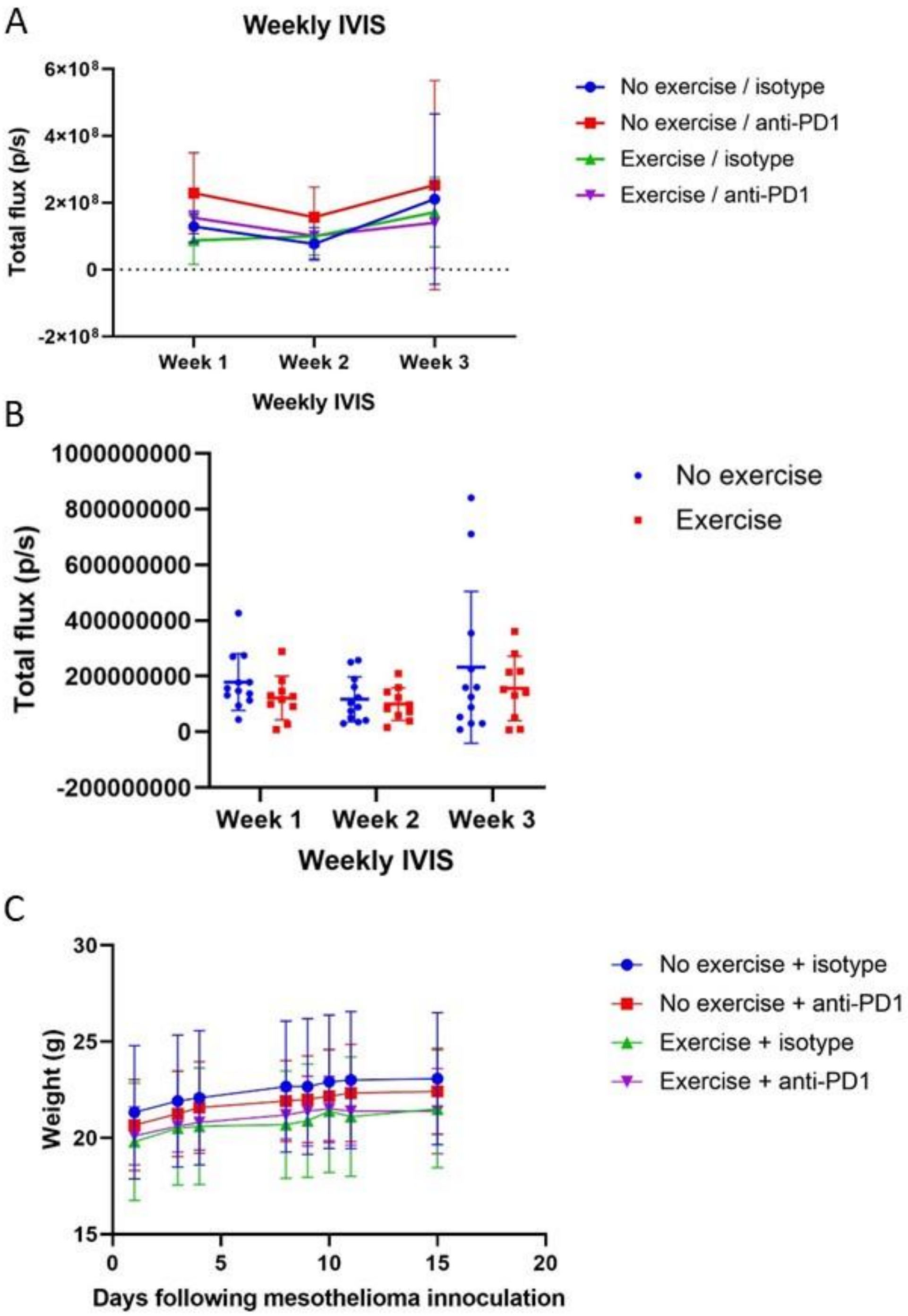


Figure 4.16: Bioluminescent intensity of mesothelioma tumours from each group from weeks 1-3

Living Image® software was used to calculate total radiance emitted from each tumour. **A.** Demonstrates the average total flux (p/s) of tumours within each treatment group from weeks 1-3 whilst **B.** Examines the differences in tumour total flux (p/s) between no exercise and exercise groups. **C.** Mice were weighed regularly through the study and all mice slowly gained/ maintained weight throughout. Shapiro-Wilk tests confirmed normal distribution of the data and so unpaired t tests were used to determine statistical significance.

4.4.2.3: Exercise causes a trend towards reduced Tregs within mesothelioma TMEs

Flow cytometry was performed on fresh tumours following end of study, with tumours manually dissociated to single cell solutions prior to staining and fixing. No statistically significant differences were seen in leucocyte subpopulations between mouse groups; however, a clear trend was noted towards reduction in Tregs within these exercising cohorts, as shown in Figures 4.17a-b (p=0.3832). This supports the Treg reduction seen within pancreatic tumours of exercising mice. Figures 4.18a-g illustrate the leucocyte subpopulations within tumours of different groups, with no statistical significances found. Similar to the flow cytometry findings from the pancreatic cancer study, a trend towards reduced CD8 T cells was seen in the exercising cohorts (Figure 4.18d).

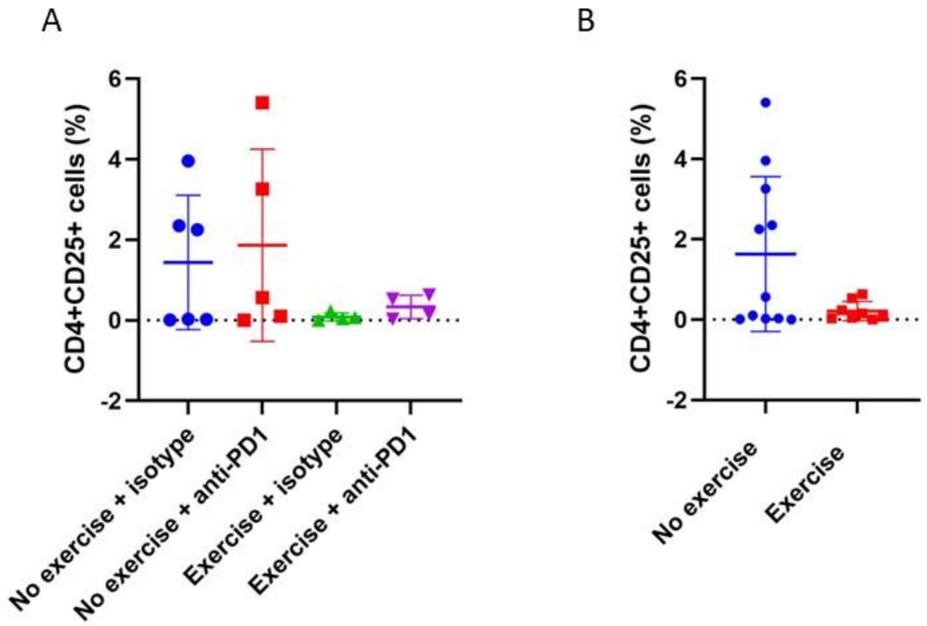


Figure 4.17: Aerobic exercise and resistance training causes a trend towards a reduction in Tregs

Flow cytometry was used to assess an immune panel including CD45, CD3, CD4 and CD25 on the mesothelioma tumours on completion of the study. **A.** A trend towards reduced CD4+CD25+ Tregs was seen with exercise and anti-PD1 and exercise and isotype. **B.** A clear trend is demonstrated towards a Tregs reduction in the exercising cohorts. One-way ANOVA and Mann-Whitney tests were used to ascertain significant differences, which were not reached.

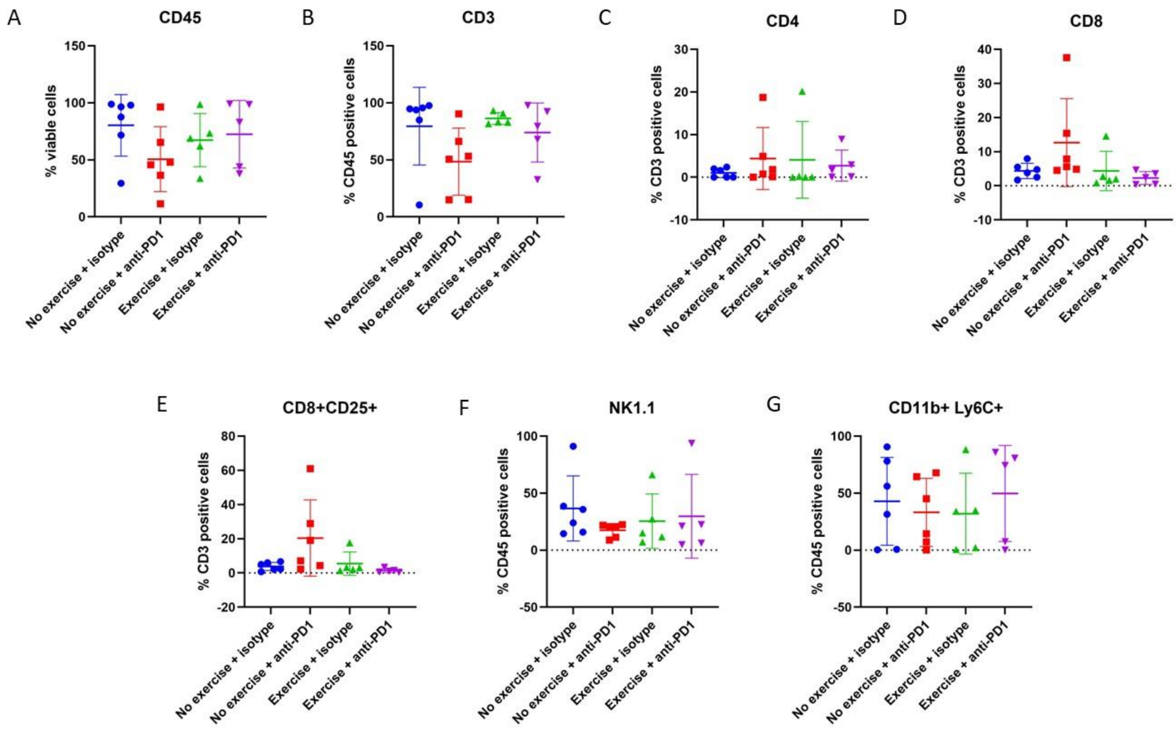
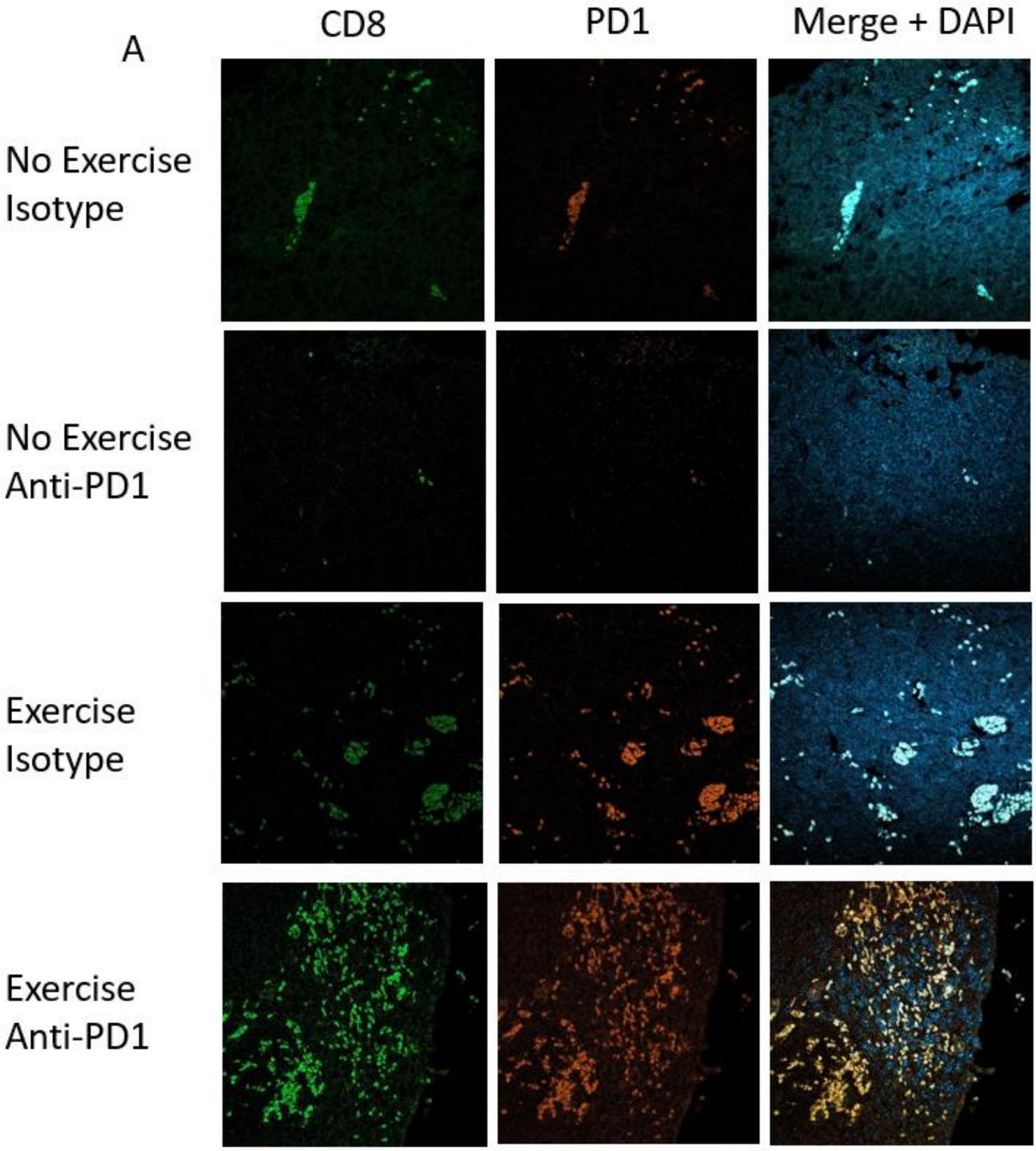


Figure 4.18: Intratumoural immune populations on flow cytometry

Flow cytometry was used to assess an immune panel including **A.** CD45, **B.** CD3, **C.** CD4, **D.** CD8, **E.** CD25, **F.** NK1.1, **G.** CD11b and Lyc6c on the mesothelioma tumours on completion of the study. A trend towards reduced CD8 and CD8+CD25+ T cells were seen in the exercising groups. One-way ANOVA and Mann-Whitney tests were used to ascertain statistical significance, which was not reached.

4.4.2.4: Exercise and anti-PD1 therapy significantly increases CD8 T cell intratumoural infiltration and upregulates PD1 expression

Similarly to the pancreatic cancer mouse study, immunofluorescent staining of mesothelioma tumours demonstrated a significant rise in CD8+ cytotoxic T cell infiltration in the exercise/anti-PD1 group compared to the no exercise/isotype (p=0.0316) and the no exercise/anti-PD1 group (p=0.0071). Figure 4.19a demonstrates the clear upregulation of PD-1 expression along with increased T cell count within the tumours of the exercise/anti-PD1 group. Figure 4.19b demonstrates the increased cytotoxic T cell infiltration and Figure 4.19c shows the increased CD8+PD1+ T cells within the tumours of the exercise/anti-PD1 group compared to the no exercise/isotype (p=0.0138) and the no exercise/anti-PD1 group (p=0.0067).


A
CD8
PD1
Merge + DAPI
No Exercise Isotype
No Exercise Anti-PD1
Exercise Isotype
Exercise Anti-PD1

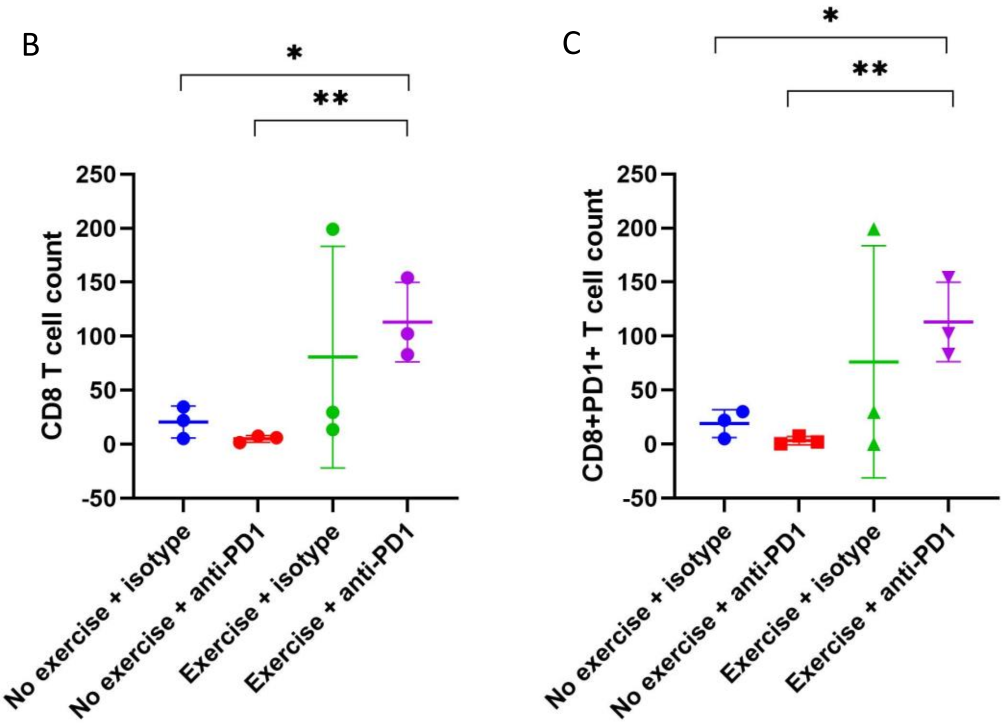


Figure 4.19: CD8 T cell and PD1 expression is upregulated within mesothelioma tumours with exercise/anti-PD1 therapy

A. Demonstrates the immunofluorescent staining tumour from the mesothelioma mouse study. Paraffin wax-embedded slides were dewaxed in xylene and hydrated sequentially with 100%, 95%, 90% and 70% ethanol. Following antigen retrieval with a heater, slides were stained with CD8-FITC (green) and PD1-PE (orange) antibodies along with DAPI. Zeiss LSM 980 Airyscan confocal was used to visualise slides at 40X magnification. Shapiro-Wilk tests were used to confirm normal distribution of the data and unpaired t tests were used to assess significance. **B.** Demonstrates differences in CD8 T cell counts for n=3 in each subgroup and **C.** Illustrates significant increases in CD8+PD1+ T cells within the tumours of exercise/anti-pd1 group, *p<0.05, ** p<p0.01

4.4.2.5: Exercise elicits a trend towards macrophage polarisation within mesothelioma TMEs

The presence of anti-inflammatory M2 macrophages within the TME perpetuates the cold immune climate, limiting the effectiveness of immunotherapy. We quantified the number of M1 and M2 macrophages within mesothelioma tumours of the different groups using immunofluorescent staining to ascertain any exercise-induced changes (Figure 4.20a). We observed a trend towards reduced M2 (F4/80+ MRC1+) macrophages in mice treated with either exercise and/or anti-PD1, although this did not reach significance (p=0.57, Figure 4.20b). M1 (F4/80 + MRC1-) macrophages were scarce within the tumours although trended towards higher levels within the exercise/anti-PD1 group (p=0.400, see Figure 4.20c).

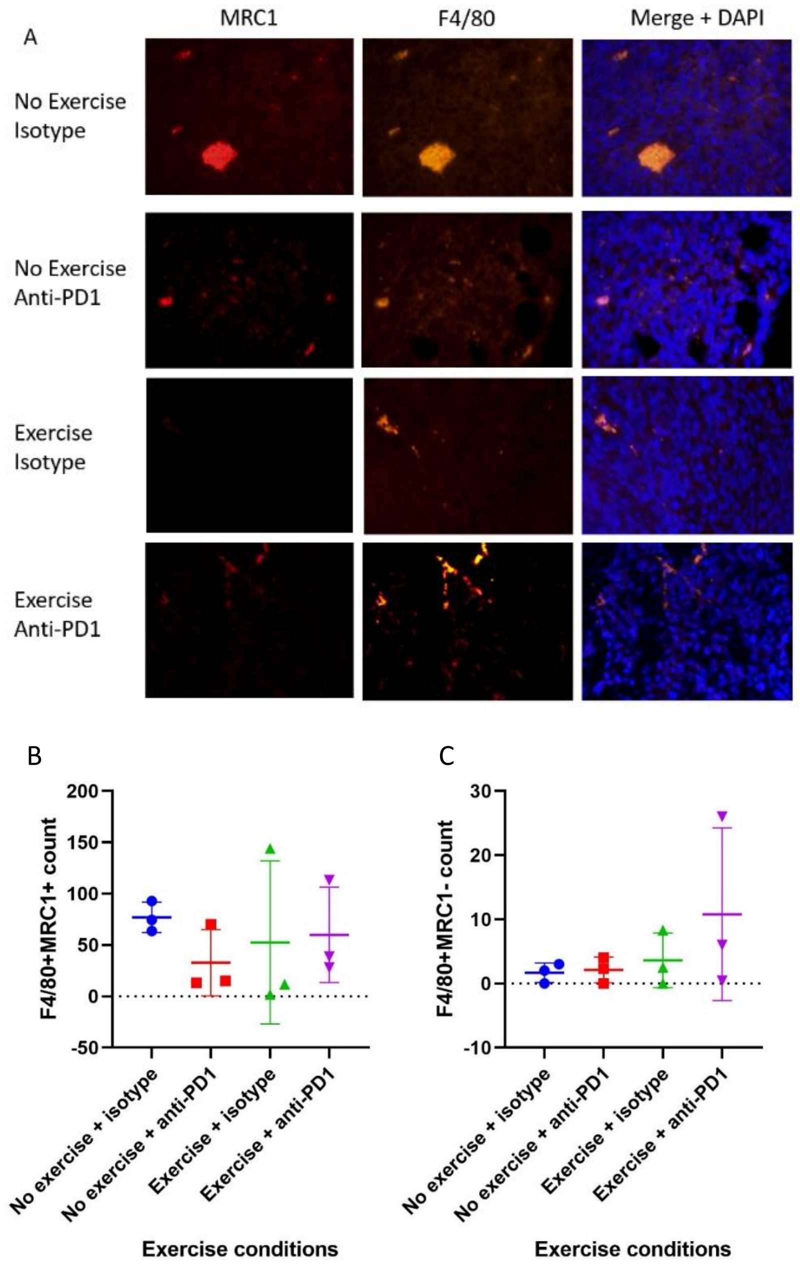


Figure 4.20: A trend towards M1 macrophage polarisation is seen within the mesothelioma tumours of mice undergoing exercise/ anti-PD1

Tumours from each group (n=3) were assessed for M1 and M2 macrophages. **A.** Demonstrates the immunofluorescent staining tumour from the mesothelioma mouse study, prepared as before, and stained with F4/80-PE, MRC1-AF647 and DAPI. All images were acquired using EVOS microscope at 20X magnification. Kruskal-Wallis and Mann-Whitney tests were utilised to assess statistical significance. **B.** Demonstrates the trend towards reduced F4/80+MRC1+ (M2 macrophages) in the tumours of mice receiving anti-PD1 and/or exercise intervention compared to the no exercise/ isotype group, although no significance reached. **C.** Demonstrates the trend towards increased F4/80+MRC1- (M1 macrophages) in the tumours of mice from the exercise/anti-PD1 group compared to no exercise/isotype group.

4.4.2.6: Exercise/anti-PD1 causes a trend towards mesothelioma tumour size reduction

All tumours were assessed on H&E slides for tumour area and necrosis. No significant changes in tumour areas were observed between the mouse groups, however once likely outliers were removed using ROUT (Robust Outlier Test) on GraphPad, a trend towards reduced tumour area was seen in the exercise/anti-pd1 group, as shown in Figure 4.21a. Interestingly, very little necrosis was observed within any of the tumours. One tumour within the no exercise/ control group with a very large tumour area on histology demonstrated some areas of necrosis, more indicative of excessive tumour size rather than treatment response. This value was removed as an outlier using ROUT, and still no significant differences in tumour necrosis were seen (Figure 4.21b).

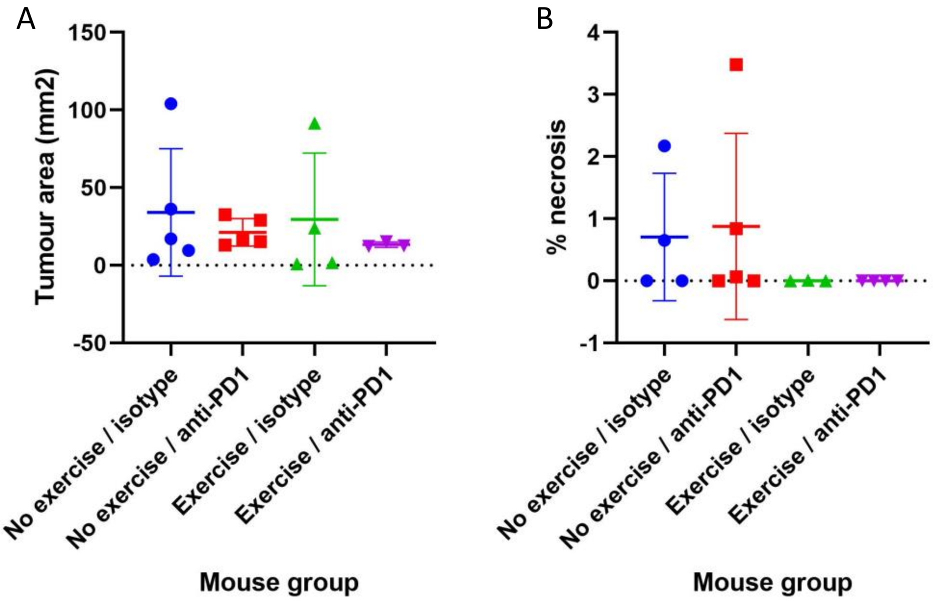


Figure 7: Mesothelioma tumour areas and necrosis from mesothelioma mouse study

Following end of study, mesothelioma tumours were harvested and placed in 4% paraformaldehyde for 1 day. Tumours were put into 70% ethanol, paraffin wax embedded, sectioned and H&E stained. SlideViewer was then used to determine **A.** Total tumour areas and **B.** Areas of necrosis within the tumours. Kruskal-Wallis and Mann-Whitney tests were used to ascertain differences between cohorts. When shown to pass the normality test with a Shapiro-Wilk test, data were analysed using parametric tests including ordinary one-way ANOVA and unpaired T-tests. ROUT was utilised to remove outliers, and data plotted in GraphPad prism v 8.0.2.

4.5: Discussion

In this chapter, we have established the feasibility of combined moderate intensity aerobic exercise and resistance training in orthotopic mouse models of pancreatic cancer and mesothelioma. This allowed us to accurately immune profile and assess tumour response to a combination of exercise and immunotherapy in these cancers. The exercise-induced changes described above were all elicited by a significantly reduced exercise duration and intensity than described in other published mouse studies (25,33,42). This suggests the required exercise dose to elicit favourable anti-cancer immune responses may be less intense than previously thought, making it more feasible to incorporate this into the clinical pathway for patients with pancreatic cancer and mesothelioma who are typically frail.

4.5.1: Cytotoxic CD8 T cells

We demonstrated a significant influx of cytotoxic CD8 T cells into both pancreatic cancer and mesothelioma tumours of exercising mice receiving at least 3 doses of anti-PD1 therapy. Lahusen et al (96) correlated increasing TME CD8 T cell concentration in pancreatic cancer with improved treatment responses and prolonged duration to treatment failure after second line therapy. The CD8 T cell enrichment was not observed in mice receiving immunotherapy alone, and explains the lack of tumour necrosis, indicating insufficient treatment response, exhibited in this group. In a similar manner, Yin et al (97) demonstrated improved responses to immune checkpoint inhibitors in mesothelioma tumours with increased CD8 T cells. Gomes-Santos et al (98) also observed a CD8 T cell influx into the breast tumours of exercising mice and using a proteomics panel, determined exercise upregulated CXCR3 ligands, CXCL9 and CXCL11, which play important roles in leucocyte mobilisation and tissue delivery. Our mouse studies illustrate exercise-induced CD8 T cell infiltration is applicable to pancreatic cancer and mesothelioma and may be pivotal in improving the immune climate of cold tumours.

4.5.2: PD-1 upregulation on CD8 T cells

Furthermore, our mouse studies demonstrated that PD-1 expression was also upregulated illustrating that exercise can elicit the activation of T cells which provide a greater target for anti-PD1 therapy. Kumagai et al determined that higher levels of

CD8+PD1+ T cells within the TME correlated with better immunotherapy responses and longer progression-free survival (PFS) in NSCLC and gastric cancer (99). Specifically in advanced mesothelioma, higher levels of CD8+PD1+ T cells with the TME was shown to be a positive predictive biomarker in patients receiving immune checkpoint inhibitors, significantly extending PFS (100).

Gustafason et al (101) demonstrated significant increases in CD8+PD1+ T cells systemically post exercise and similarly to our findings, Bay et al (102) observed exercise-induced increases in CD8+PD1+ T cells within a melanoma mouse model, with enhanced IFN γ secretion, indicating activation rather than exhaustion. Therefore, these changes within the TMEs of pancreatic cancer and mesothelioma could correlate to improved survival outcomes for these patients.

It is important to note the disparity between flow cytometry findings and immunofluorescent staining of paraffin-wax embedded tumour slides in terms of CD8 T cell quantification. Although, fresh tumours were prepared for flow cytometry on the day of harvesting, there was a great degree of cell death, which influenced the flow cytometric analysis. Single cell solutions were prepared from tumour samples by manual dissociation for flow cytometry. With immunofluorescent staining, immune cells are depicted in their exact location within the tumour tissue. Therefore, the immunofluorescent staining findings of increased CD8 T cell and PD1 upregulation in tumours from exercising mice are more likely to be representative of TME modulations induced by exercise.

4.5.3: MDSCs and Tregs

It is not only the lack of effector immune cells which creates the ‘cold’ immune climate, the presence of immune tolerant MDSCs and Tregs provides a shield against immune recognition and promotes tumour propagation. We have shown significant exercise-induced reductions in these immune components within the pancreatic tumour which works synergistically with CD8 T cell infiltration to enhance anti-cancer immunity. A clear trend towards MDSC/ Treg reduction in the mesothelioma model was seen although significance not reached.

The mechanism behind exercise-induced leucocytosis is thought to be secondary to catecholamine-mediated activation of B2-adrenergic receptors, which take advantage of exercise-induced increased cardiac output and mobilisation of effector immune cell pools within lymphoid tissue into the systemic vasculature (103). Studies have demonstrated that it is not the absolute number of MDSCs/ Tregs that is reduced with exercise, but the proportion within the total immune cell populations reduce systemically (104). Our study has been able to correlate these systemic changes with intratumoral immune modulation within pancreatic cancer and mesothelioma. The TME of pancreatic cancer is notoriously hostile and resistant to immune cell/ drug delivery with tortuous tumour vasculature perpetuating a hypoxic environment. Despite this, we have demonstrated that systemic increases of effector cells elicited by exercise are able to penetrate the fibrous pancreatic tumour. As previously mentioned, studies have shown exercise-induced normalisation of tumour vasculature in peri-operative pancreatic cancer patients (14), a separate mechanism that allows transformation of the cold tumour environment.

Future work would include examining further immune cell populations within mesothelioma tumours on histology including Tregs and MDSCs. To further explore the mechanisms behind exercise-induced responses, proteomic analysis of tumour lysates would allow the accurate and sensitive quantification of a range of cytokines and myokines which could be implicated in the anti-cancer response.

4.5.4: NK cells

There was a distinct lack of NK cells present within the pancreatic tumours despite our previous findings of significant mobilisation and systemic release of NK cells with exercise. NK cells are vital elements of innate immunity and are usually the initial responders, therefore as intratumoural NK cell infiltration was only examined at the end of the study, this may account for the reduced numbers noted compared to CD8 T cells. Furthermore, NK cells may have provided a key protective role in inhibiting / limiting metastases in these models. NCR1 is receptor involved with NK cell activation and has been shown in melanoma and lung mouse models to be crucial in suppressing metastases when compared to NCR1-deficient mice (99). Interestingly, primary tumours were unaffected by the lack of NCR1 indicating the main utility of exercise-induced NK cell systemic release may be to control and eliminate metastatic disease. Future work should include exploring NK cell mobilisation and activation (both systemically and intratumourally) within these cancer models and in patients with cancer.

4.5.5: Exercise reduces metastatic potential

The pilot study demonstrated that treadmill running significantly reduced the burden of liver micro-metastatic disease in pancreatic cancer. The anti-PD1 pancreatic cancer mouse model confirmed significantly reduced liver micro-metastatic disease with exercise, irrespective of drug treatment. This highlights that exercise is the key contributor to limiting the metastatic potential of pancreatic cancer. No liver, lung or spleen metastases were noted within the mesothelioma mouse model.

Systemic exercise-induced NK release may be responsible for inhibiting the development and progression of metastases (as discussed in the section above). Preventing the migration and invasion of pancreatic cancer cells into distant tissues could significantly improve survival outcomes for patients at all stages of pancreatic cancer.

Future work could include reviewing systemic NK cell cytotoxicity by quantifying interferon gamma/ granzyme B using ELISA kits as well as measuring factors involved in EMT, including MMP 2/9, E-Cadherin and vimentin by immunohistochemistry.

4.5.6: M1 and M2 macrophages

Trends towards a reduction in pro-tumorigenic M2 macrophages were seen in tumours undergoing either or both interventions with a trend towards increased anti-tumorigenic M1 macrophages seen within the exercise/anti-PD1 group. However, this should be interpreted cautiously because of the small numbers of M1 macrophages seen and the small sample size. Kwon et al demonstrated a skew to M1 macrophage polarisation with a complementary M2 macrophage depletion in a breast cancer mouse model with moderate-intensity exercise (30-minute treadmill running 5 days/week) (105). However, conflicting evidence within the literature has shown an exercise-induced upregulation of the M2 phenotype in colorectal cancer liver metastases (106). The exercise intervention used in the latter study consisted of 1 hour of treadmill running 5 days/week for 4 weeks, which is significantly longer than most studies and highlights the importance of exercise dose-finding within these studies.

Limitations included the small sample size (n=3). Further exploration of the macrophage profile within the tumours is warranted, including increased sample numbers and evaluation of other M1 markers, such as inducible nitric oxide synthase (iNOS).

4.5.7: Exercise increases tumour necrosis

We demonstrated a higher degree of necrosis within the pancreatic tumours of exercising mice compared to non-exercising mice. Pancreatic tumours from the exercise/anti-PD1 group exhibited the largest areas of necrosis indicating treatment response. This supports findings from Martin-Ruiz et al., using a combination aerobic exercise/resistance training within a non-small cell lung cancer mouse model, where the highest necrotic index was seen in their exercise/nivolumab (anti-PD1 therapy) treatment group (33). Tumour necrosis denotes a lack of viable tumour tissue and within the pancreatic cancer study, correlated to areas of CD8 T cell infiltration, implicating these cells as key executors of the anti-tumour immune response. Importantly, tumour necrosis does not unequivocally indicate treatment response and must be taken in context with viable tumour tissue histologically.

Within the mesothelioma mouse study, generally minimal necrosis was seen across all tumours. Larger tumours within the control group exhibited some areas of necrosis, indicating perpetuation of an hypoxic environment when the vasculature could not meet the demands of the exponentially proliferating cancer cells (107). Therefore, the trend towards reduced viable tumour area seen within the exercise/anti-PD1 group, once the outlier was removed, and reduced bioluminescent intensity of pancreatic tumours in the exercising cohorts on IVIS, warrants further review in larger pre-clinical and clinical studies. Due to the direct administration of the aggressive AB-1 mesothelioma cells into the peritoneal lining, tumours grew quickly and mice from control groups did reach human endpoints around day 15. Therefore, future work could utilise a refined protocol, with reduced number of AB-1 cells used during cancer implantation. This would give time for the combination of exercise and immunotherapy to exert effects on the tumour.

4.5.8: Conclusion

We successfully carried out orthotopic mouse models in pancreatic cancer and mesothelioma, showing feasibility of a combined aerobic exercise and resistance training programme alongside anti-PD1 therapy. We have shown that exercise modulates the TMEs of both these cancers by enhancing CD8+PD1+ T cells and reducing immune tolerant MDSCs and Tregs. Exercise exerts inhibitory effects on the primary and metastatic tumours in pancreatic cancer and demonstrates that a combination of exercise and anti-PD1 therapy could be a promising treatment option for these cancers.

Chapter 5

Exercise to Boost Immunity in advanced Cancer: feasibility of combined aerobic Exercise and resistance training for Patients with advanced mesothelioma and pancreatic cancer (BICEP)

5.1: Introduction

Alongside our exercise studies in healthy volunteers and mice, we intended to carry out a feasibility study of an exercise programme in patients with advanced mesothelioma and pancreatic cancer undergoing first line palliative anti-cancer treatment. There are no specific exercise guidelines for patients affected by cancer who are undergoing anti-cancer treatment. To deliver patient-centred exercise prescriptions, we require a better understanding of the FITT criteria that achieves reproducible and effective outcomes in cancer care (4). Graded exercise supervised by specialists will identify feasible exercise activities we can incorporate into clinical practice which elicit favourable immune responses. Once we can establish feasibility of effective exercise training in advanced mesothelioma and pancreatic cancer patients, this will allow larger studies exploring the efficacy of exercise as an adjunct to immunotherapy in these patients.

We will be carrying out a feasibility study with the primary objective being to assess the practicality and acceptability of a structured aerobic exercise and resistance training regime for patients with advanced mesothelioma and pancreatic cancer who will be undergoing first line palliative systemic cancer treatment with immunotherapy (mesothelioma) or chemotherapy (pancreatic cancer). These advanced cancer populations generally consist of older age adults with multiple co-morbidities and a degree of frailty. Therefore, a feasibility study to assess the practicalities of an exercise regime alongside palliative treatment would be vital to provide safe, effective and realistic exercise doses to use as an immunotherapy adjunct.

Our exploratory analyses will include extensive immune profiling (leucocyte subtype and cytokine characterisation) of exercising participants at different stages of the exercise training (which will gradually build up under expert exercise-specialist review as per patients' capabilities). We can therefore assess the appropriate level of exercise in these cancer patients that elicits the immune response needed. This study will provide further information for a larger, robust randomised trial assessing the efficacy of exercise as an intervention in different cancer cohorts and its potential ability to enhance immunotherapy responsiveness and widen the spectrum of tumours that benefit from immunotherapy.

Study design, protocol and all associated documents were completed and ethics approval was obtained during the course of this PhD. Funding is now being sought for the feasibility study which will follow on from the PhD.

5.2: Hypotheses

1. A combination of aerobic exercise and resistance training is feasible alongside palliative anti-cancer treatments in patients with advanced mesothelioma and pancreatic cancer.
2. Moderate-intensity exercise elicits a favourable systemic anti-cancer immune profile in patients with advanced mesothelioma and pancreatic cancer, including raised NK and CD8 T cells and reduced MDSCs and Tregs.

5.3: Aims and Objectives

1. Complete the Integrated Research Application System (IRAS) and receive ethics approval from the Health Research Authority (HRA) and favourable opinion from the Research Ethics Committee (REC).
2. Design a study protocol
3. Design a patient information sheet, lay summary, consent form and GP letter
4. Complete applications to secure funding for the feasibility study

5.4: Ethics

An ethics application was prepared for a feasibility study in human patients via IRAS (IRAS project ID 321450). This has been granted HRA approval and favourable opinion from the REC (REC reference 24/YH/0093). The ethics application required clear methodology, study rationale as well as input from all research team members including collaborators at the Advanced Wellbeing Research Centre (AWRC). The application has required Medical Physics Expert (MPE) and Clinical Radiation Expert (CRE) reviews. This is due to the use of images and reports of standard of care CT scans that patients recruited into the study would be having. Even though no further scans or radiation are required by being involved in the study, the MPE and CRE reviews were required and have been performed. The ethics application has passed the radiation assurance self-managed process. The IRAS form is located in the supplementary documents (appendix A).

5.5: Timeline

Figure 1.1 demonstrates the timeline from feasibility study set-up to implementation of NHS-funded exercise programmes within Oncology practice.

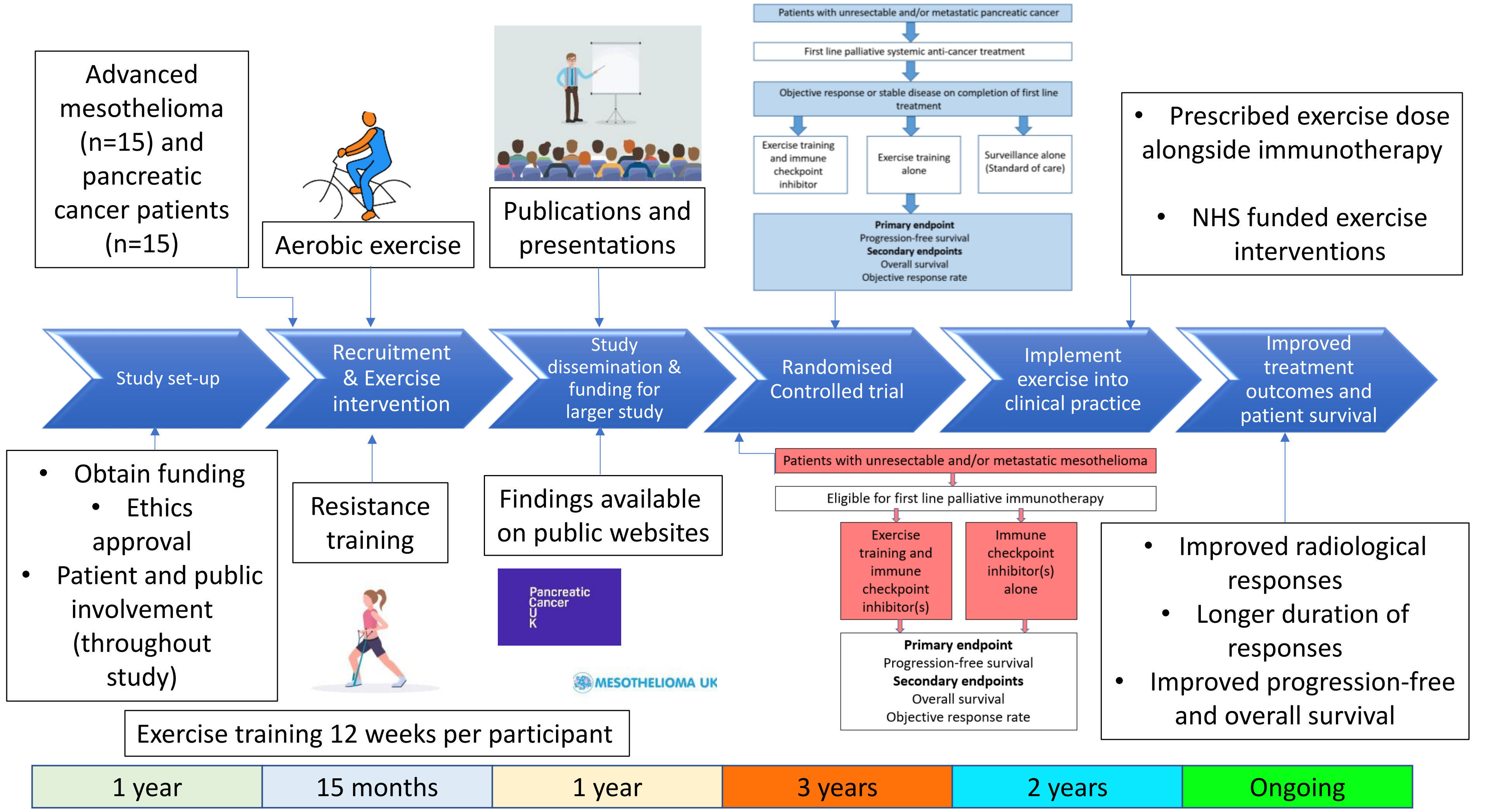


Figure 8: Timeline for clinical trials

Timeline outlining the steps involved in feasibility study set-up, dissemination of information and development of a randomised controlled trial to demonstrate effectiveness of exercise and immunotherapy combined in pancreatic cancer and mesothelioma. Subsequent incorporation of exercise prescriptions in Oncology practice would then lead to improved outcomes for patients with cancer (108).

5.6: Study Protocol

The study protocol was reviewed and amended multiple times through the Clinical Research and Innovation Office (CRIO) and underwent two independent scientific reviews to gain approval and final acceptance through the IRAS process. The finalised study protocol is provided below.

STH RESEARCH DEPARTMENT

Project details

1. Investigator details

Chief Investigator: Dr Brindley Hapuarachi, Clinical Research Fellow in Translational Oncology, Specialist Registrar in Medical Oncology, MBBS, BSC, MRCP. Division of Clinical Medicine, University of Sheffield, Beech Hill Road, Sheffield, S10 2RX.

Co-investigator: Professor Munitta Muthana

Co-investigator: Professor Sarah Danson

Co-investigator: Professor Jonathan Wadsley

Co-investigator: Professor Caroline Dalton

Co-investigator: Dr Liam Humphreys

2. Sponsor details

Sheffield Teaching Hospitals (STH) NHS Foundation Trust

3. Project title

Exercise to **B**oost **I**mmunity in advanced **C**ancer: feasibility of combined aerobic **E**xercise and resistance training for **P**atients with advanced mesothelioma and pancreatic cancer (BICEP)

4. Project Reference Numbers

Local Reference: STH22549

IRAS Number: 321450

Registry Reference:

5. Protocol version number and date

Version 1.0 21/02/2024

6. Signatures of Chief Investigator and Sponsor*

7. STH Directorate affiliation

Specialised Cancer Service

Table 5.1: Key Trial Contacts

Chief Investigator	Brindley Hapuarachi sbhapuarachchi1@sheffield.ac.uk
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Sponsor Contact	Ms Grace Cole grace.cole1@nhs.net

5.6.1: Sponsorship and Funding

5.6.1.1: Sponsor

STH have responsibility for the sponsor duties of this project following the UK Policy Framework. STH will be the Data Controller.

5.6.1.2: Funder

A grant application is being considered for funding via MRC Gap fund for early-stage development of new healthcare interventions.

5.6.2: Trial Steering Committee

We will set up a Trial Steering Committee, consisting of members of the research team including collaborators at the Advanced Wellbeing Research Centre (AWRC) and patient and public involvement (PPI) members. This Trial Steering Committee will meet virtually to discuss updates and progress of the study.

The activities the Trial Steering Committee will advise on include, supporting grant applications, study recruitment, exercise protocol adherence and any study amendments. The main focus would be on patient safety and satisfaction. Recruitment will be carefully monitored by the Trial Steering Committee and contingency plans considered if recruitment is slower than anticipated.

5.6.3: Research question

Is exercise a feasible intervention alongside systemic anti-cancer treatment in patients with mesothelioma and pancreatic cancer?

5.6.4: Abstract

5.6.4.1: Background

Immune checkpoint inhibitors (ICIs) demonstrate effectiveness in many cancer settings, although over 60% of patients with cancer do not benefit from ICIs (1). Mesothelioma shares characteristics of hot and cold tumours due to its lymphocyte rich TME including effector immune cells (Natural Killer, CD8 T cells) and immune tolerant cells (MDSCs and Tregs) (10). Mesothelioma displays mild clinical responses to ICIs with some benefit compared to conventional chemotherapy regimens, although the prognosis remains poor (8,109). On one end of the spectrum, classically 'cold' tumours such as pancreatic cancer, exhibit a lack of immune cells and low immune activity (5). Research amongst cancer patients indicates exercise improves general health, may prolong survival as well as reduce cancer-related fatigue and support anti-cancer immunity through multiple mechanisms (4,110). Exercise-induced leucocytosis is where there is an instantaneous systemic release of leucocytes following acute exercise (21). The use of exercise as medicine requires an understanding of the appropriate dose which is highly dependent on the FITT principles (Frequency, Intensity, Time, and Type) and this is crucial for generating desired outcomes (4,111). Exercise could potentially transform the tumour immune climate from cold to hot and could be a potential adjunct to immunotherapy.

The aim of this trial is to determine the feasibility and acceptability of moderate intensity interval aerobic exercise during immunotherapy treatment in patients with mesothelioma and chemotherapy in patients with pancreatic cancer.

5.6.4.2: Methods

The trial is a prospective feasibility trial conducted in Sheffield. Recruitment will include thirty patients receiving first line palliative immunotherapy for advanced, unresectable or metastatic mesothelioma and patients receiving first line systemic anti-cancer treatment for pancreatic cancer. Patients will attend the AWRC for a supervised exercise session once a week to include aerobic exercise along with an unsupervised weekly exercise session for 3 months. Blood samples will be collected at baseline and then monthly for 3 months, pre and post the supervised exercise session. Cytokine, myokine and immune cell concentrations will be analysed using flow cytometry, cytokine bead-based multiplex immune assays and proteomics panels to full profile any exercise-induced changes.

5.6.4.3: Analysis

Non-parametric analyses will be used to determine any statistically significant difference in immune and inflammatory biomarkers with Wilcoxon signed-rank tests where matching is possible. Feasibility of exercise will be dictated by the adherence rate looking at how many of the organised exercise activities were undertaken (112). Acceptability will be shown by the ratio of those recruited to the trial to those approached as eligible. The attrition rate will be assessed by the ratio of dropouts from the trial to those registered at the start. Safety of exercise will be assessed by documentation of directly related adverse events. Disease response will be assessed as per RECIST 1.1 criteria on standard of care CT scans. Lung function tests will be performed at regular time points to assess aerobic capacity and the effects of exercise on forced expiratory volume and maximal oxygen capacity.

5.6.5: Design

Feasibility trial

5.6.6: Setting

The trial will be based at two sites. Participants will be recruited at STH and will include patients (n=30) from the local area receiving first line palliative immunotherapy for advanced, unresectable, or metastatic mesothelioma as well as patients receiving first line systemic anti-cancer treatment for pancreatic cancer. Participants will attend the AWRC, part of Sheffield Hallam University (SHU) for a supervised exercise session once a week to include aerobic exercise and resistance training along with an unsupervised weekly exercise session for 3 months.

5.6.7: Objectives and Outcome Measures

5.6.7.1: Primary Objective

To determine the feasibility of exercise during systemic anti-cancer treatment in patients with advanced mesothelioma or pancreatic cancer.

5.6.7.2: Secondary Objectives

i. To determine the safety of exercise during systemic anti-cancer treatment in patients with advanced mesothelioma or pancreatic cancer.
ii. To determine the acceptability of exercise during systemic anti-cancer treatment in patients with advanced mesothelioma or pancreatic cancer.
iii. To determine the effect of exercise on immune cell subpopulations and activation
iv. To determine the effect of exercise on cytokine/ myokine levels in the blood
v. To determine the effect of exercise on radiological response or progression.
vi. To determine the effect of exercise on lung function.
vii.

5.6.7.3: Primary Outcome Measure

Adherence rate: How many of the organised exercise activities were undertaken

5.6.7.4: Secondary Outcome Measures

i. The number of directly related adverse events.
ii. Acceptability rate: the ratio of those recruited to the trial to those approached as eligible.
iii. Attrition rate: the ratio of dropouts from the trial to those registered at the start.
iv. Peripheral blood mononuclear cells (PBMC) in the blood at defined timepoints (see table below). Immune cell responses will be determined by an increased in cytotoxic immune components (Natural Killer cells / CD8 T cells) and a decrease in immunosuppressive immune cells (regulatory T cells).
v. The levels of cytokines/ myokines in the blood at defined timepoints (see table below)
vi. Evidence of radiological response or progression as per RECIST 1.1 reporting of CT scans at defined timepoints (see table below).
vii. Lung function as per results of lung function tests at defined timepoints (see table below).

<u>Table 5.2: Table of Objectives and Outcome Measures</u>

Objectives	Outcome Measures	Timepoint Measured At

To determine the feasibility of exercise during systemic anti-cancer treatment in patients with advanced mesothelioma or pancreatic cancer.	Adherence rate: The number of organised exercise activities that were undertaken.	At cessation or completion of the study
To determine the safety of exercise during systemic anti-cancer treatment in patients with advanced mesothelioma or pancreatic cancer.	The number of directly related adverse events.	Throughout the study
To determine the acceptability of exercise during systemic anti-cancer treatment in patients with advanced mesothelioma or pancreatic cancer.	Acceptability rate: the ratio of those recruited to the trial to those approached as eligible.	At cessation or completion of the study
	Attrition rate: the ratio of dropouts from the trial to those registered at the start.	At cessation or completion of the study
To determine the effect of exercise on immune cell subpopulations and activation	PBMCs in the blood. Immune cell responses will be determined by an increased in cytotoxic immune components (Natural Killer cells / CD8 T cells) and a decrease in immunosuppressive immune cells (regulatory T cells).	Baseline, then 4-weekly pre- and post- exercise interventions and at each exercise intensity increment for 12 weeks and then at completion of the 12-week exercise intervention
To determine the effect of exercise on cytokine/	The levels of cytokines/ myokines in the blood	Baseline, then 4-weekly pre- and post- exercise

myokine levels in the blood, which are known to promote immune system activity?		interventions and at each exercise intensity increment for 12 weeks and then at completion of the 12-week exercise intervention.
To determine the effect of exercise on radiological response or progression.	Evidence of radiological response or progression as per RECIST 1.1 reporting of CT scans	Baseline and at 12 weeks
To determine the effect of exercise on lung function.	Lung function as per results of lung function tests	Baseline, 12-week exercise intervention.

5.6.8: Participants

5.6.8.1: Inclusion Criteria

- Patients about to start or who are undergoing first line palliative immunotherapy for advanced, unresectable, or metastatic mesothelioma or patients about to start or who are undergoing first line palliative chemotherapy for advanced, unresectable, or metastatic pancreatic cancer.
- Age over 18 years old
- Histological or cytological diagnosis of mesothelioma or pancreatic cancer.
- ECOG Performance status 0-1 (to be assessed by clinician)
- Ability to provide informed consent

5.6.8.2: Exclusion Criteria

- Prior treatment with systemic anti-cancer treatment
- More than one primary cancer
- Uncontrolled brain or bone metastases
- Patients who have progressed on first line palliative immunotherapy for advanced, unresectable, or metastatic mesothelioma.
- Patients who have progressed on first line palliative chemotherapy for

advanced, unresectable, or metastatic pancreatic cancer.

- Patients with active co-morbidities that would prevent or limit their participation in the exercise intervention
- Age below 18 years old
- No histological or cytological diagnosis of mesothelioma or pancreatic cancer.
- ECOG Performance status of 2 or more
- Unable to provide informed consent

5.6.8.3: Sample Size:

15 patients with mesothelioma and 15 patients with pancreatic cancer. This a feasibility trial and therefore, a power calculation was not performed. The sample size was chosen on agreement within the research team and with a Medical Statistician to provide information on feasibility measures.

5.6.8.4: Recruitment and Consent

All eligible patients that attend mesothelioma and pancreatic cancer clinics at Weston Park Hospital and peripheral clinics across South Yorkshire over the duration of recruitment will be approached to discuss the study. As all patients with advanced pancreatic cancer undergoing palliative systemic treatment in South Yorkshire will be seen and treated at Weston Park Hospital, we will be recruiting from the representative population. Patients with advanced mesothelioma will be seen by clinicians from Weston Park Hospital either centrally or at peripheral clinics in South Yorkshire. Therefore, patients from different geographical and socioeconomic groups will be approached across South Yorkshire.

Patients will be provided with a written patient information sheet (PIS) along with a consultation with the investigator. Information relayed within the PIS, the reasoning behind the trial and what it will involve will be discussed. Patients will have sufficient time to ask any questions. If the patient is interested, then contact details will be provided and patient will subsequently sign a consent for the trial at a later consultation.

Where needed, interpreters will be used to ensure there are no issues with language barriers at all steps within the study. Participants will also be able to bring their family

/ friends to the AWRC for each exercise activity. The study intervention involves travel to the AWRC. Transport costs to and from the AWRC will be covered. Snacks, drinks, and refreshments will also be provided. Therefore, there will be no financial burden on participants, and they will be able to receive the benefits of supervised tailored exercise regime at no cost. The populations affected by these cancers will be at a more advanced age and therefore careful consideration of transport, supervision and tailoring of any exercise activity to the individual will take place and this will all be emphasised to patients during recruitment.

Patients across Yorkshire will have varying access to exercise facilities and resources. Many patients may not have an opportunity to gain input and supervision from exercise specialists due to multiple reasons including socioeconomic differences and language barriers. This study will provide this expertise and supervision to many patients who may otherwise not be able to gain the benefits of exercise as they either don't have the means to do so or don't feel safe to exercise alone.

This study will define a safe exercise regime that patients with advanced mesothelioma and pancreatic cancer can perform during treatment and in the long term. This includes activities within an exercise facility and also home-based exercises performed with Fitbits. Therefore, this project will offer invaluable advice regarding what exercise patients with advanced cancer can do at home on their own, providing health benefits to patients that may not be able to access exercise facilities and reducing cancer-related health inequalities.

5.6.8.5: Early Discontinuation/ Withdrawal of Participants

Patients can withdraw at any point if they wish. On withdrawal, patients would be able to continue with standard-of-care treatment as they wish. Participants may require study withdrawal by the Investigator in special circumstances such as loss of the participant to provide consent or concerns with regards to delivery and performance of the exercise intervention due to the participant’s health condition.

5.6.9: Analysis including statistical methods, where appropriate

Quantitative data will be analysed using non-parametric tests given the limited sample size (30 patients). Wilcoxon signed-rank tests will be conducted to assess whether any differences in paired data from individuals pre- and post- exercise are statistically significant. When pairing is not possible, Mann-Whitney tests will be performed. For repeated measures at 4-weekly intervals with more than 2 values, non-parametric ANOVAs (performed on ranks) will be used and graphically represented.

The feasibility of the study will be assessed by the adherence rate by calculating how many of the organised exercise activities were performed (112). Any incidents directly arising from exercise activities will be documented. Acceptability will be shown by the ratio of those recruited to the trial to those approached as eligible. The attrition rate will be assessed by the ratio of dropouts from the trial to those registered at the start.

5.6.10: Intervention

Exercise activities: The exercise programme will include one weekly supervised exercise session at the AWRC and one weekly unsupervised exercise session.

The supervised session will include cycling on an exercise bicycle, at moderate intensity with periods of rest, and resistance training. An exercise specialist present will be monitoring vital signs and tailoring the session to ensure the level of exercise is appropriate and safe. The following points describe the FITT (Frequency, Intensity, Type and Time) principles that will be used as a plan for the exercise programme.

Frequency: Twice weekly, one supervised and one unsupervised session a week for 12 weeks.

Intensity: Moderate intensity aerobic interval exercise (step-wise build up to 60% maximum heart rate) combined with 1-3 sets of 6-12 Repetition Maximum (RM) resistance training tailored to individual capabilities.

Type: Aerobic interval exercise (cycling) and resistance training (3 exercises: chest press, biceps, and leg curl)

Time: Aim to build to 4 x 4-minute cycling at 60%, max heart rate (HR) with 3 minutes active recovery. 3 sets with 2 minutes per set – around 20 minutes resistance training. Intensity/duration of exercise will be adjusted to individual capabilities.

Participants will be given further advice regarding the unsupervised exercise session from the specialist, which will take place in their own home. Participants will be given wearable devices (Fitbits) to track their HR, monitor activity and sleep. Participants will be advised to wear this during their home exercise and also continuously for 1 week at the beginning of the exercise programme and 1 week at the end. Participants will also have the option to wear the devices all the time. This will allow us to account for physical activity/ exercise outside of the study. These weekly sessions will be arranged so that they do not interfere with any of their cancer appointments or treatments. These sessions will last over a 12-week period.

5.6.11: Trial Assessments

Baseline information will be collected including age, sex, race/ethnicity, medical conditions and medications, normal level of physical activity, type and stage of cancer and information regarding previous cancer treatments. A full medical history including physical examination, baseline measurements of height and weight and general observations will be performed. The trial will be timed around the standard of care CT scans, no participants will have a CT scan at baseline specifically for this trial.

Participants will have their vital signs, including HR and oxygen saturations, measured during the supervised exercise sessions. They will also be given a wearable device to wear during their home exercise sessions to assess their vital signs, such as HR.

Lung function tests will be performed at baseline and at the end of the trial (at 12 weeks, or earlier if participants leave the trial sooner).

Bloods will be taken for immune profiling and cytokines/ myokines at baseline, during the intervention period at defined time points (see appendix D) and at the end of the trial (at 12 weeks, or earlier if participants leave the trial sooner).

Participants will have CT scans every 3 months as standard of care. If they agree to take part in the trial, they will have the same number of scans as if they were not in the trial.

We will also assess quality of life using EQ-5D questionnaires.

See appendices C-D for trial flow chart and schedule of assessments.

5.6.12: Sample Storage and Analysis

Blood samples will be stored and analysed within the laboratories in the University of Sheffield (UoS). Leukocytes and serum will be prepared and either tested immediately or stored frozen until analysis. Flow cytometry will be performed on whole blood samples from anticoagulated EDTA-blood tubes to define and characterise different leucocyte subpopulations. Serum will be analysed to assess changes in cytokine profiles. Samples will be stored for use in future research and learning and destroyed 5 years after the study has ended. This will be optional for participants to consent to. If participants decide not to consent to this, their samples will be destroyed at study completion.

Samples will be stored in the Biorepository at University of Sheffield.

5.6.13: Safety Reporting

Trained specialists will supervise exercise sessions at the AWRC, and any adverse event (AE) related to the exercise intervention will be clearly documented and reported back to the research team. For any AEs that occur during the unsupervised exercise session, patients will have a clear out of hours contact number to report these.

Any Serious Adverse Events (SAEs) that are directly related to the study's exercise activities and events that are not expected are to be communicated with the REC within a 15-day time period.

5.6.13.1: Expected Occurrences

The following may be expected to occur:

5.6.13.1.1: Exercise Intervention

- Injury to musculoskeletal system including muscle/joint pain, injuries to tendons/ligaments and skeletal fractures.
- Cardio-respiratory symptoms including breathlessness and chest pain.

5.6.13.1.2: CT Scans

- Contrast allergy / hypersensitivity.
- Long-term effects of radiation including small increase in the potential risk of other cancers.

5.6.13.1.3: Lung Function Tests

- Dizziness, shortness of breath and cough.

5.6.13.1.4: Venepuncture

- Discomfort, bruising, bleeding, infection around needle site.

5.6.14: Assessment and Management of Risk

Exercise is safe and generally recommended for the majority of the population, any potential risks will be limited due to supervision with specialists.

The risk of falling/ sustaining an accident would be minimised during the supervised session when a trained exercise specialist with experience in working with people with cancer is present to acknowledge individual patient limitations. There will be standardised exercise protocols in place that can be graded according to the patients' needs and abilities. This will then dictate advice on exercise interventions to be performed unsupervised. The specialist will monitor the patient carefully throughout the session. All exercise protocols have an associated risk assessment that has assessed and mitigated any risks associated with the delivery of physical activity sessions specifically for people living with cancer.

The risk of over-exertion with exercise in a cohort that does not do regular exercise will be minimised by ensuring that patient limitations are addressed and reviewed by the exercise specialists to ensure the participants do not have any cardiovascular, respiratory, or musculoskeletal concerns.

5.6.15: Quality Control

Standard operating procedures (SOPs) are in place from the Clinical Research and Innovation Office (CRIO).

5.6.16: Monitoring

The trial will be monitored in accordance with CRIO policy.

5.6.17: Regulatory Compliance

Medical Physics Expert and Clinical Radiation Expert took place due to the involvement of standard-of-care CT scans.

5.6.17.1: Clinical Radiation Expert review:

Study design:

Determine the feasibility and acceptability of exercise during immunotherapy treatment in patients with mesothelioma as well as in a non-immunotherapy treated cohort of patients with pancreatic cancer.

15 patients with advanced mesothelioma (on or due to start first line palliative nivolumab and ipilimumab) – median overall survival 18.1 months.

15 patients with advanced pancreatic cancer (on or due to start first line palliative chemotherapy) – median overall survival 11.1 months.

Objectives (relevant to imaging/radiation exposure):

a) CT imaging at regular time points will be performed to assess evidence of radiological response or progression as per RECIST 1.1.
b) Prepare a clinical trial protocol: Combining immunotherapy with exercise to improve the responsiveness to immunotherapy in a larger cohort.

Imaging Procedure requirements:

CE CT CAP at baseline and at 3 months, reported according to RECIST 1.1

The exposures are in line with standard clinical care in terms of frequency a participant will not receive additional exposure as a result of participation.

Additional reporting requirements – RECIST 1.1 is above standard clinical care and will require 3d lab input/funding.

Summary:

The 2 CE CT CAP exposures are part of standard clinical care not additional, both exposures are justifiable on that basis.

No additional ionising radiation is required for this study, all CT scans will be performed as standard of care. Additional RECIST 1.1 reporting will be performed on these CT scans, and this data will be reviewed as part of the study to assess radiological response alongside the exercise intervention with their standard of care treatments.

5.6.18: Project Plan with Timescale

Feasibility trial to run over 18 months (January 2026 to July 2027). The end of recruitment date will be December 2026. The study end date is defined by when the last patients has had their last visit. The last patient's last visit will be planned for March 2027.

5.6.18.1: Declaring End of Trial Prematurely

Criteria for electively stopping the trial or other research prematurely include failure to recruit and safety concerns with exercise intervention.

5.6.19: Expertise of the Researcher and Associated Team

Dr Brindley Hapuarachi: Medical Oncology Registrar, research fellow and PhD student. He has presented internationally and received the ASCO merit award for his oncology research.

Professor Munitta Muthana: Professor in Immuno-Oncology has a long-standing track record of immunotherapy work and reprogramming tumours to be more immune responsive.

Professor Sarah Danson: Professor and Honorary Consultant Medical Oncologist in melanoma and early phase treatments, the national ECMC Network Lead and the NIHR National Speciality Lead for Early Phase Research in Cancer.

Professor Jonathan Wadsley: Honorary Professor, Consultant Clinical Oncologist, academic director for the directorate of Specialised Cancer Services, clinical director of the Sheffield Cancer Clinical Trials Centre.

Professor Caroline Dalton: Associate Professor, Sheffield Hallam University, leads the Health and Disease group at the Biomolecular Sciences Research Centre with over 15 years' experience in studying exercise interventions. She co-leads the 'Living Well with Chronic Disease' theme with Dr Liam Humphreys.

Dr Liam Humphreys: Senior Research Fellow with a track record for designing and delivering exercise interventions for people with cancer.

Together we have over 20 years' experience in designing and leading clinical trials, including early phase and pancreatic cancer trials.

5.6.20: Patient and Public Involvement (PPI)

We understand the importance of involving patients and their families at all stages in setting up and carrying out this trial. Members of the research team have attended a PPI workshop, building connections with underserved communities. PPI members from Weston Park Hospital and with the Experimental Cancer Medicine Centre (ECMC) conference have reviewed study documents including the lay summary and PIS, allowing us to make improvements and to see whether patients are on board with what we hope to gain from this trial

This trial has been presented to dedicated PPI panels at the Mesothelioma UK Research Centre and Pancreatic Cancer UK including outlining the feasibility trial, background, aims, methodology and hopeful impact and translational endpoints. We have received very positive feedback and interest regarding this trial from the patients and their families affected by mesothelioma, specifically the concept and that patients would like to be involved in doing something themselves to help their cancer treatment. A patient with mesothelioma attributed being physically active during her immunotherapy to improved tolerability and treatment response. Families were thankful that this research was being done to improve responses for other patients.

PPI has helped shaped the design of this trial with the inclusion of resistance training alongside aerobic exercise within the biweekly exercise sessions. One patient advised they lost weight, specifically muscle, during the course of their treatment. With appropriate dietician input and strength training, muscle loss could be combatted as well as provide further immune enhancement with myokine release.

Following initial PPI discussions, we have been asked back multiple times to discuss updates with the PPI panels. This has helped with the practicalities of improving patient experience in the trial, including allowing family and friends to attend with the patient and to ensure appropriate facilities and amenities are available for them at the AWRC.

5.6.21: Dissemination

The findings from this trial will be shared with all members of the patient and public involvement groups attached to this project via email, with a plan to disseminate in peer-reviewed journals and academic conferences. We will ask all trial participants if they want to be told the trial results and if so, we will send them a trial summary via email. We will also contact Mesothelioma UK and Pancreatic Cancer UK to allow this research to be available freely on their websites for the public.

5.6.22: Taking the Work Forward

We plan to utilise the information from this trial to power and design a robust, randomised control trial to assess the effects of exercise on local tumour and systemic immunity and response to systemic anti-cancer treatments in mesothelioma and pancreatic cancer.

5.7 Discussion

5.7.1: Rationale for feasibility study

The study has the potential to meet the clinical need of improving / expanding treatment options for these cancer groups and allow patients to receive the benefits from immunotherapy which have improved survival outcomes in other cancers. Exercise would be a safe, cost-effective intervention that could be incorporated into NHS clinical practice, improving tolerability of treatment and reducing healthcare economic burden. One key competitive advantage is that a new drug would not have to be administered alongside immunotherapy, leading to inevitable cost savings from avoiding drug manufacturing, purchasing and delivery.

The CHALLENGE trial has demonstrated that regular moderate-high intensity aerobic exercise in patients undergoing curative treatment for colorectal cancer leads to enhanced survival (113). Further studies have provided an increasingly strong basis for the utility of exercise in the peri-operative setting in breast cancer (114,115). These survival benefits have not yet been investigated in the advanced cancer populations.

There have been feasibility studies exploring exercise in advanced cancer populations and a systematic review looked at 25 studies with patients with advanced cancer, largely lung cancer (116). Acknowledging the large variance in patient demographics from cancer diagnosis, stage and metastatic burden, high adherence rates were demonstrated across a range of exercise intensities and importantly, with programmes lasting more than 4 weeks (116). This review highlights the need to explore specifics of exercise dosing in different cancers. Our study aims to address the lack of feasibility and safety data of graded exercise regimes alongside palliative care treatment in advanced pancreatic cancer and mesothelioma. Furthermore, this study will be able to provide novel insights into exercise-induced immunity using detailed immunological profiling in patients with cancer.

5.7.2: Implementation of exercise into clinical practice

To demonstrate translatability into clinical practice, we have discussed how we can implement an exercise prescription alongside treatment. The combination of home-based exercise and specialist-supervised activities would be key to improve patient engagement and allow maximal benefit across the range of patients with different capabilities and well-being. We have discussed collaborating with industrial partners to develop an app to aid in the monitoring and recording of advised exercise activities. Furthermore, collaboration with community facilities such as St Luke's hospice, may allow NHS-funded supervised exercise sessions to support the implementation of exercise.

Exercise in cancer is a growing field, however specific anti-cancer mechanisms and modulation on tumour immunity correlating to improved clinical outcomes needs to be evaluated with this feasibility study and a larger trial. To the best of our knowledge, exercise within advanced pancreatic cancer and mesothelioma and the consequent immune responses have not been reviewed within the context of clinical trials. Exercise as pre-habilitation in peri-operative pancreatic cancer has been explored and shown to be feasible (117). This is promising as these patients undergo major surgery (Whipple's procedure) and exhibit high levels of morbidity and so can mirror the relative frailty of advanced pancreatic cancer patients. This study will play a vital part in allowing these cancer groups to achieve benefits from immunotherapy and is required to de-risk the intervention prior to larger study.

5.7.3: Challenges to study set-up

5.7.3.1: Funding applications

The following grant applications were made to try to secure funds for the study. The last application can be found in the supplementary appendix B.

1. National Institute for Health and Care Research (NIHR) Research for Patient Benefit (RfPB)

2. Pancreatic Cancer UK (PCUK) Research Innovation Fund (RIF)

3. Yorkshire Cancer Research (YCR) More Life to Live (MLTL) Scheme

I was Principal Investigator (PI) for the first two applications but the YCR MLTL Scheme specified that the PI must have a substantive contract with the UoS and therefore, I was unable to be PI despite providing the major contribution for the application.

Unfortunately, we were not successful with the NIHR RfPB or PCUK RIF applications. We received positive feedback with regards to study concept, design and patient and public involvement (PPI). The RfPB scheme required greater benefit to patients to what a feasibility study in these cancer groups could offer. The RIF scheme required higher risk and greater benefit as well. The YCR MLTL application proceeded past internal review with very favourable comments and went to the final stages of the application with positive comments and reviews from a world cancer panel. Due to the nature of the feasibility study, it did not quite fit the remit for the MLTL scheme so was unsuccessful as well (see appendix B for the YCR MLTL application).

With the feedback from the grant applications, we have amended the application and study protocol to explicitly state that exercise will be graded in a step-wise approach with exercise physiologist input and tailored to the individual, working up to a moderate intensity level for both aerobic exercise and resistance training. This will allow the extensive immune profiling at different time points and intensities of the exercise programme which in turn will allow the characterisation of a safe, effective exercise dose which can be utilised in the larger studies exploring the effectives of an exercise-immunotherapy combination in pancreatic cancer and mesothelioma.

We have discussed with the MRC Gap Fund Programme Manager to confirm eligibility of this study to the programme which we aim to submit in November 2025. We have also consulted the Translational Research Manager from UoS who will help co-ordinate this project application in line with MRC expectations. If successful with this funding, we plan to start this study in 2026. Following this PhD, I will apply for a Clinical Lectureship to be able to carry on this research and lead this study.

5.7.3.2: Delays in ethics application submission

To submit the IRAS ethics application, the form along with all study documents required over 10 iterations with delays in review from the CRIO with each version.

The study protocol required 2 independent scientific reviews which delayed the project for a further 2 months. Furthermore, due to the use of standard-of-care CT imaging within the study, radiation assurance, MPE and CRE reviews were required introducing further delays. This process took 18 months to achieve the necessary sign-offs for IRAS submission. Once submitted, favourable REC opinion and HRA approval was achieved within 4 weeks.

Most delays were out of my control due to the busy schedules of everyone in the review process. However, for future IRAS applications involving imaging, I would ensure the self-managed process for radiation assurance along with MPE/ CRE reviews were performed earlier in to prevent delays further down the line.

5.7.3.3: Limitations

There were a number of limitations to the applications which may have hindered successful funding. Firstly, at the time of the initial applications, limited pre-clinical evidence was available from our group to support the rationale for the feasibility study. At the end of this PhD project, there is now considerable pre-clinical evidence from our vitro and mouse studies, supporting the utility of exercise as an immune enhancer and adjunct to immunotherapy in pancreatic cancer and mesothelioma. This will strengthen future applications.

The research team consists of experts within Immunology, Oncology and Exercise Physiology and has been commended by several review panels. However, in order to enhance the breadth of expertise and build connections throughout Yorkshire, our research team now include Dr Cindy Forbes, a senior researcher from the University of Hull, who leads the YCR-funded research of home-based exercise in breast cancer patients. With further discussions with the AWRC, we will also be including a healthy psychologist and dietician into the research team to better support patients holistically throughout the study.

Finding the suitable funding stream for a feasibility study of an early intervention has also proven to be challenging. Despite initial discussions with programme managers, the main barrier to successful funding has been the inability to demonstrate direct, quantifiable patient benefit from the study. This is not something we could do at this stage of the feasibility study and so, we identified the MRC Gap Fund Scheme as a more suitable scheme due to its focus on developing early interventions prior to a larger study. We also now have a translational research project manager on board to help co-ordinate this application.

5.7.4: Conclusion

This feasibility study will provide the vital step for defining the exercise dose in advanced pancreatic cancer and mesothelioma patients that is acceptable and sufficient to elicit the immune responses described in detail in the previous results chapters. This will then lead to a randomised controlled trial exploring the benefit of an exercise-immunotherapy combination in these cancers. Despite challenges, the study application has been amended and strengthened incorporating a multi-disciplinary team with a wide breadth of expertise. The eventual goal would be to incorporate NHS-funded exercise activities as an adjunct to immunotherapy within the clinical pathway for these patients.

Chapter 6

General Discussion

6.0: Discussion

Immunotherapies, such as ICIs, have significantly improved treatment efficacies and survival statistics in classically hot tumours such as melanoma (5). However, many cancers, such as pancreatic cancer, remain resistant to ICIs largely due to their immune-deplete tumour microenvironments. Immunotherapy has modest effects in cancers which lie in middle of the cold-hot spectrum, including mesothelioma, where benefits can be seen compared to standard chemotherapies, but prognoses remain poor (8,109).

Strategies to reprogramme immune-resistant tumours to be able to reap the benefits from immunotherapy have included combining ICIs with chemotherapy as seen in advanced triple negative breast cancer and lung cancers (118). However, combination treatment can often be significantly toxic limiting tolerability and dose intensity, consequently reducing potential effectiveness. Furthermore, chemotherapy-immunotherapy combinations have not been shown to be sufficiently beneficial in pancreatic cancer and mesothelioma to warrant NHS funding.

Patients with these advanced cancers tend to be older adults with varying degrees of co-morbidity dependant on stage and burden of disease. First line palliative treatment currently consists of multi-agent chemotherapy (FOLFIRINOX) for pancreatic cancer and dual immunotherapy (nivolumab and ipilumumab) for mesothelioma. Only a minority of these patients are fit for these treatments and unfortunately, many patients would not be well enough for any systemic treatment at diagnosis. Patients often do not complete the treatment course either due to unacceptable toxicity or disease progression. 5-year survival rates for all stages of both of these cancers remains poor at 5-10% (6,119). There is a clear unmet need to improve management and responses in these cancers to correlate with improved survival outcomes.

This research set out to determine the effect of high intensity interval aerobic exercise on systemic immunity in healthy volunteers and evaluate any exercise-induced changes in cancer cell viability, migration and apoptosis in mesothelioma and pancreatic cancer; tumours at different parts of the cold-hot spectrum. This then supported the exploration of the effects of a combination of exercise and anti-PD1 therapy in pancreatic cancer and mesothelioma mouse models. Furthermore, we designed and set-up a feasibility study to explore practicalities of implementing an exercise programme in patients with these advanced cancers. With our mouse and human studies, we consciously chose to downgrade the intensity of exercise from high to moderate to support the literature with regards to benefits in cancer and to increase the likelihood of acceptance and adherence of exercise within an advanced cancer population.

To our knowledge, our mouse studies are the first to demonstrate the benefits of a combined aerobic exercise and resistance training in pancreatic cancer and mesothelioma, with a reduced intense aerobic activity of 10-15 minutes of treadmill 4 days/ week compared to previous studies which have utilised 30 minutes of treadmill running 5 days/week (25,33,42). This is essential to finding the optimal dose of exercise which can provide the significant immune benefits whilst also being feasible in an elderly / frail advanced cancer population.

6.1: NK cells may limit metastatic potential

This research has shown that exercise could provide a pivotal part in boosting systemic and intratumoural immunity in these cancers to allow ICIs to work better. Firstly, we have shown that aerobic exercise was able to stimulate an instantaneous mobilisation and release of NK cells in all healthy volunteers (see Chapter 3 Figure 3.2). However, NK cell numbers were not increased with exercise over 3 weeks within the TMEs of pancreatic cancer and mesothelioma (see Chapter 4 Figures 4.7 and 4.18). It is important to highlight the differences in intensity, duration and type of exercise between these studies as well as the potential varied immune responses between mice and humans. In the healthy volunteer's study, blood samples were collected immediately following an acute high intensity aerobic exercise compared to the mouse studies where a combined moderate intensity and resistance training programme took place. Intratumoural NK cells were measured after 3 weeks and not directly after an exercise activity which is when NK cell upregulation is likely to occur.

As previously discussed, NK cells are key factors of innate immunity and exercise-induced NK cell mobilisation may play a greater role in prevention and control of metastatic disease than primary tumour response (99). Therefore, whilst NK cell numbers were not upregulated in our mouse experiments, we do observe a significant reduction in micrometastatic liver disease within the exercising cohorts. To further explore this, future work could include measurement of systemic NK cell count along with activation and cytotoxicity assays from these mouse models.

However, it is unlikely that this suppression of metastases is solely due to NK cell activity. Our in vitro findings showed significantly reduced migration of pancreatic cancer cells when cultured with post-exercise conditioned serum. Myokines, such as irisin and IL-6, are released into the blood stream from contracting muscles during exercise and will likely be present in abundance within exercise-conditioned serum (120). Irisin has been implicated as a key disrupter to EMT by increasing E-cadherin and reducing vimentin, effectively anchoring tumour cells to their position and preventing mobility (121). Future work would include the characterisation of a panel of cytokines/myokines that could be eliciting these anti-cancer effects. Following our unsuccessful attempt with cytokine bead arrays to quantify common cytokines within participants' sera, we could instead utilise proteomics panels, such as O-Link, to look at a wide range of cytokines with great sensitivity to assess differences in pre- and post-exercise conditioned serum. This has been factored in with the costs for a feasibility study to allow extensive immune profiling of patients with advanced pancreatic cancer and mesothelioma which has not been reported before in the literature and would be vital information to move forward with a larger trial exploring the efficacy of an exercise-immunotherapy combination in these cancers.

It is still in debate whether aerobic exercise or resistance training is the greater catalyst for myokine mobilisation (120). It is likely there isn't a clear answer to this, but with the known health benefits for both exercise forms with regards to cardiorespiratory functioning and musculoskeletal conditioning, a combination of aerobic exercise and resistance training would be ideal to explore in clinical studies. Furthermore, from PPI activities performed with the set-up of the feasibility study, patients had enquired about the addition of resistance training to the study protocol. Discussions revealed positive experiences for patients who undertook muscle strengthening exercises during their cancer treatment. This included the ability to maintain their weight and reduce muscle loss, keeping them feeling active and generally well. Weight loss has been shown to correlate with poor prognoses in many cancers and is a significant issue in patients with pancreatic cancer (122).

Specialist nutritional support and supplementation would be essential alongside exercise to combat cancer cachexia. Following these discussions, it was confirmed with the exercise specialists at AWRC that resistance training will be included alongside aerobic exercise within the study protocol and specialist dietician input would be provided for all participants.

6.2: CD8 T cells implement enhanced tumour response

No significant differences were determined in CD8 T cell populations within exercise in the healthy volunteer's study post exercise. However, we did observe cytotoxic CD8 T cells and PD1 expression were significantly upregulated with exercise/anti-PD1 therapy within tumours from both pancreatic cancer and mesothelioma mouse models. In contrast to NK cells, our findings suggest that measuring CD8 T cells immediately after a single bout of exercise may not provide sufficient time to elicit a response. This could explain the significant benefits seen in the mouse models with a 3-week exercise programme with repeated exercise activities stimulating augmented CD8 T cell mobilisation and distribution into tumours.

Areas within the pancreatic tumours enriched with CD8+PD1+ T cells correlated with significantly increased tumour necrosis. This implicates activated cytotoxic CD8 T cells, upregulated with exercise/anti-PD1 therapy, in causing response within the tumours and eliminating viable tumour.

6.3: Reduced Tregs and MDSCs produce 'hotter' immune climates

Whilst exercise-induced leucocytosis is an established phenomenon, exercise-induced intratumoural immune effects and the systemic and intratumoural contraction of immune tolerant cells in pancreatic cancer and mesothelioma are less well described. Our findings reveal exercise significantly reduces Tregs both systemically in healthy volunteers and within pancreatic cancer TMEs and trends towards a decrease in mesothelioma TMEs. Our study also demonstrated a significant reduction of MDSCs with exercise in the pancreatic cancer model, which further supports the transformation towards a hotter TME, by attenuating immune tolerance and this supports findings from other studies (79).

The presence of Tregs and MDSCs is a major barrier to the effectiveness of immunotherapy and studies have explored ways to target these immune populations, such as with daclizumab, an anti-CD25 monoclonal antibody (123). In order to enhance our study findings, we could look into CD4+CD25+FOXP3+ cells within our tumours with immunofluorescent staining, to more accurately define Tregs and confirm changes seen with flow cytometry.

6.4: Macrophage polarisation to M1 phenotype

In our mesothelioma mouse study, we explored exercise-induced phenotypic modulation of TAMs. Trends towards reduced ‘pro-tumour’ M2 macrophages and increased ‘anti-tumour’ M1 macrophages were seen within tumours of mice in the exercise/anti-PD1 therapy group. Within our healthy volunteers study, we demonstrated a systemic reduction in CD14+ monocytes following an acute high intensity aerobic exercise. Circulating monocytes migrate to tumours driven by chemokines, including C-C motif ligand 2 (CCL2) (124). Using a range of cytokines, e.g. IL-10 and TGF-β, M2 macrophages quickly establish themselves as the dominant cell type within cold tumours (124). This research highlights that exercise-mediated systemic monocyte reduction could lead to intratumoural M2 macrophage depletion. In a breast cancer preclinical model, a reduction in M2 macrophages within the tumour was noted with low-moderate intensity exercise (125). Myostatin, an exercise-induced myokine, was implicated as the driving force for this benefit by diminishing JAK1/STAT6 macrophage- signalling which is crucial to monocyte/macrophage recruitment to the tumour (125,126).

Due to time constraints, we were unable to explore this within the pancreatic cancer mouse samples. Future work would include more detailed macrophage characterisation of both tumour models, including other M1 markers, such as iNOS.

6.5: Limitations

6.5.1: Healthy volunteer's study

High intensity was measured subjectively using the rate of perceived exertion. Although this is an established scale within exercise studies, it is open to individual interpretation and may introduce bias. Objective measures such as maximum heart rate and VO2 max would more accurately define intensity levels and allow standardisation across particpants. Due to lack of resources this was not carried out within the healthy volunteer's study. Importantly, this has been addressed within the design of the feasiblity study, and resources at the AWRC would allow the objective measurement and titration of intensity of all particpants. Furthermore, the healthy volunteer's study explored a single bout of exercise at a single timepoint after exercise. Evidence shows that the immune response to exercise is dynamic and changes over time (24). Exploring the effect of exercise training across different timepoints is a limitaiton we hope to address with our feasiblity study. Regular immune profiling throughout 12 weeks should give us a clearer picture of the potential benefits of exercise alongside cancer treatment.

Another limitation, which is inherent to most exercise studies, is that recruitment of participants will likely be skewed to those more willing to adhere to exercise activities, likely already undertaking a degree of regular exercise which may not be representative of cancer populations. Recruitment strategies to ensure equality, diversity and inclusion have been extensively reviewed within our research team and PPI groups and connections with diverse community groups, including the Deep End Research Alliance, will hopefully counteract this.

Unsuccessful cytokine bead arrays meant we were unable to determine the precise factors within the exercised serum that inhbited migration and enhanced apoptosis. As discussed before, further research with proteomics could explore a wide range of cytokines/myokines that could be implicated in these anti-cancer effects.

6.5.2: Mouse studies

Aggressiveness of the AB-1 cell line within the peritoneal cavity meant control mice showed signs of discomfort at day 15 and tumours had grown significantly on IVIS review indicating the need to end the study on day 16. This may have limited the possible effectiveness of the combination of exercise/anti-PD1 therapy in modulating the TME immune climate and its ability to elicit tumour necrosis. Refinement would include further study with mesothelioma innoculation with reduced AB-1 cell count. Furthermore, due to lack of ultrasound equipment and expertise within the department, we were unable to perform intrapleural implantation of mesothelioma. This would have allowed a more accurate representation of the most common site of mesothelioma in clinical practice and an intrapleural mouse model may be an area to consider for future research.

6.6: Future work

Following the feasibility study, next steps will be designing and setting up a larger randomised controlled trial exploring the effectiveness of an exercise and immunotherapy combination against the standard of care. For mesothelioma, standard of care for first line palliative treatment would be dual immunotherapy with nivolumab and ipilumumab, therefore exploring this combination with exercise can be done within the first line treatment setting. However, for pancreatic cancer, immunotherapy is currently not available under the NHS and so an exercise/ immunotherapy combination would be best examined in the maintenance setting following either response or stable disease to first line palliative treatment (108). Current practice includes a surveillance period following first line treatment until progression. At this point, it they are well enough, patients may be offered second line treatment. Within this clinical trial, patients would be randomised to a combination exercise/ immunotherapy arm or standard-of-care ‘watch and wait’ after first line treatment. To warrant amendment of current clinical guidelines and NHS-funding, a survival benefit will need to be demonstrated. A larger trial would hope to answer questions regarding progression-free and overall survival. If survival outcomes are deemed to be superior with exercise/immunotherapy, a clear pathway of incorporation of exercise prescription and services into clinical practice is required. Therefore, involvement of community facilities that could support supervised exercise activities would be vital along with self-help guides/ records in the form of electronic applications to support successful implementation of exercise alongside immunotherapy.

A basket study design would be ideal in the setting of a larger trial, where the same intervention can be applied to many different cancers. Ideally, we would want to expand the benefits gained with exercise to all patients with cancer.

To build on the systemic immune profiling of blood from exercising participants of the feasibility study, a larger trial should include tumour biopsies to assess tumour response and the TME and correlate findings from our mouse studies. Given the large numbers required for this study, proteomics panels can be used allowing high throughput analysis of large numbers of cytokines/ immune checkpoints/ immune markers.

6.7: Conlusion

This research highlights the need for exploration of the use of exercise in pancreatic cancer and mesothelioma patients to boost anti-cancer activity, modulate TMEs towards hotter immune climates and enhance ICI responsiveness.

6.7.1: Key findings

1. **A single bout of high intensity exercise elicits an instantaneous systemic release of NK cells with complementary reduction in Tregs**
2. **Exercise-conditioned serum reduces migration and enhances apoptosis of pancreatic cancer and mesothelioma cells in vitro**
3. **Exercise-conditioned serum and HSV1716 work synergistically to reduce mesothelioma cell viability in vitro**
4. **Exercise, irrespective of drug treatment, modulates the pancreatic cancer TME by reducing MDSCs and Tregs**
5. **Exercise elicits a significant increase in CD8 T cell infiltration with PD1 upregulation within the TMEs of pancreatic cancer and mesothelioma**
6. **Exercise and anti-PD1 combination leads to the greatest pancreatic tumour response with highest degree of necrosis**
7. **Exercise limits metastatic disease burden in pancreatic cancer**

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Appendix A: IRAS application (relevant sections)

OVERVIEW OF THE RESEARCH

To provide all the information required by review bodies and research information systems, we ask a number of specific questions. This section invites you to give an overview using language comprehensible to lay reviewers and members of the public. Please read the guidance notes for advice on this section.

A6-1. Summary of the study. Please provide a brief summary of the research (maximum 300 words) using language easily understood by lay reviewers and members of the public. Where the research is reviewed by a REC within the UK Health Departments' Research Ethics Service, this summary will be published on the Health Research Authority (HRA) website following the ethical review. Please refer to the question specific guidance for this question.

Immunotherapy is a type of cancer treatment that helps the immune system fight cancer. It works by helping immune cells recognise and attack cancer cells. Immunotherapy has shown to be an effective treatment for many different types of cancers, however there is still work to be done to achieve better and longer responses to immunotherapy.

Exercise has been shown to improve general outcomes in cancer patients. Studies show that exercise can boost the immune system and so could potentially make immunotherapy work better and increase the range of cancers immunotherapy can provide benefit for.

Immunotherapy is used in patients with advanced mesothelioma, however better and longer responses are warranted for this group of patients. Immunotherapy is not currently beneficial for patients with advanced pancreatic cancer and small, short-lived benefits can sometimes be seen with chemotherapy. However, when combined with immunotherapy, exercise may have the potential to improve the attack against cancer including hard to treat tumours such as pancreatic cancer.

We have chosen to look at these two cancers due to their varying responses to immunotherapy. This research will aim to find standard exercise activities that these patients find acceptable, realistic and something that they can continue in the long term. We will recruit patients from Weston Park Hospital, Sheffield with advanced mesothelioma and pancreatic cancer who are currently receiving or due to start anti-cancer treatment. We will be collecting blood samples from these patients monthly before and after exercise, as well as assessing the response of the cancer to treatment using medical imaging (Computed Tomography). The exercise activity will last up to 12 weeks. We also hope to gain a better understanding of the effect of exercise on the immune system and therefore, the effect it has on how well anti-cancer treatment works.

A6-2. Summary of main issues. Please summarise the main ethical, legal, or management issues arising from your study and say how you have addressed them.

Not all studies raise significant issues. Some studies may have straightforward ethical or other issues that can be identified and managed routinely. Others may present significant issues requiring further consideration by a REC, HRA, or other review body (as appropriate to the issue). Studies that present a minimal risk to participants may raise complex organisational or legal issues. You should try to consider all the types of issues that the different reviewers may need to consider.

This study will involve recruitment of patients from NHS clinics at Weston Park Cancer Centre, Sheffield who have advanced mesothelioma or pancreatic cancer who are going to receive first line palliative systemic anti-cancer treatment.

The patients will then undergo two exercise sessions per week for 12 weeks alongside receiving their anti-cancer treatments. One of these weekly sessions will be supervised by exercise specialists at the Advanced Wellbeing Research Centre (AWRC), the other weekly session will be unsupervised and they will be given information with regards to aerobic exercise and resistance training to be done unsupervised at home along with a wearable device (Fitbit) to monitor their heart rate and step count.

One issue that will be addressed is organising the supervised exercise session at the AWRC around the participants' standard of care treatments and appointments at Weston Park Cancer Centre.

Explicit informed written consent from patients along with detailed patient information sheets are required.

Another issue includes patients with these advanced cancers (mesothelioma and pancreatic cancer) undergoing first line palliative systemic anti-cancer treatment may have significant side effects and see a drop in their performance status making their attendance and completion of exercise activities difficult. Exercise interventions will be safely performed with patient limitations being addressed. Also, exercise specialists with long-standing track records of working with people with cancer, will be supervising one weekly session and ensuring a standardised, safe and acceptable exercise intervention takes place, individually catering exercise interventions to the participants ability along with advise with regards to unsupervised exercise session.

In Sheffield the Mesothelioma UK Research Centre (MURC) hosts a dedicated Patient and Public Involvement (PPI) Panel, to ensure research is in line with the needs and priorities of patients and their family members. This PPI panel has reviewed the study with positive feedback and helpful comments and advice.

A7. Select the appropriate methodology description for this research. Please tick all that apply:
Feasibility/ pilot study

A10. What is the principal research question/objective? Please put this in language comprehensible to a lay person.
To determine the feasibility of exercise during systemic anti-cancer treatment in patients with advanced mesothelioma or pancreatic cancer.

A11. What are the secondary research questions/objectives if applicable? Please put this in language comprehensible to a lay person.
i. To determine the safety of exercise during systemic anti-cancer treatment in patients with advanced mesothelioma or pancreatic cancer.
ii. To determine the acceptability of exercise during systemic anti-cancer treatment in patients with advanced mesothelioma or pancreatic cancer.
iii. Does exercise boost immune cell activation in cancer patients?
iv. To determine the effect of exercise on cytokine/ myokine levels in the blood
v. To determine the effect of exercise on radiological response or progression.

vi. To determine the effect of exercise on lung function.

A12. What is the scientific justification for the research? Please put this in language comprehensible to a lay person.

Immunotherapy is a type of cancer treatment that helps the immune system fight cancer. It works by helping immune cells recognise and attack cancer cells.
Immunotherapy has shown to be an effective treatment for many different types of cancers, however there is still work to be done to achieve better and longer responses to immunotherapy. Not all cancer patients respond to immunotherapies as their tumours don't contain active immune cells. These patients may benefit from other anti- cancer treatments such as chemotherapy, however some cancers such as advanced pancreatic cancer are particularly hard to treat, have small, short-lived benefits from chemotherapy and no response to immunotherapy.
Therefore, there is an unmet need to improve the efficacy of anti-cancer treatment for this population.

This research investigates whether exercise can boost the immune system in cancer patients and therefore, improve response to immunotherapy in cancers where it is already used and also make immunotherapy beneficial against cancers that are hard to treat and currently do not respond to immunotherapy.

Exercise has been shown to improve general outcomes in cancer patients. In animal models, exercise has also been shown to have a positive impact in boosting cells of the immune systems in the blood along with in the local area around the cancer. This has in turn shown to reduce the size of different types of tumours in mice. Animal studies have also shown that when exercise is combined with immunotherapy, tumours in mice (including difficult to treat tumours such as pancreatic cancer) shrink to a greater degree when compared to immunotherapy alone.

Further research is required to give a better understanding on the role of exercise and how it might improve cancer care, especially with immunotherapy. We hope to add to this research and understand the role of exercise in optimising a person's immune system to give immunotherapy the best chance of working.

The main purpose of this study is to look at whether a structured exercise programme, with the help of exercise specialists, in patients with advanced mesothelioma and pancreatic cancer is practical and acceptable for these patients whilst undergoing anti-cancer treatment. Patients with advanced mesothelioma are chosen because only around 40% of these patients who receive immunotherapy respond to the treatment. Therefore, using this study if we can confirm a structured exercise programme is feasible in these patients whilst undergoing immunotherapy, we can then go on to look at a larger number of patients in a robust controlled trial looking at the effects of exercise in improving immunotherapy response.

Patients with advanced pancreatic cancer are chosen because immunotherapy currently does not work well in this group of patients. Therefore, if the study can confirm that a structured exercise programme is safe, practical and acceptable to this group of patients whilst receiving chemotherapy (which is the only option for anti-cancer treatment for this group of patients), then a further trial with a larger number of patients can look into combining exercise with immunotherapy to see if this improves how well immunotherapy works in pancreatic cancer.

This research is vital to establish the practical and acceptable exercise regimes for patients with advanced mesothelioma and pancreatic cancer who can be significantly limited by respiratory difficulties, cancer cachexia and frailty. This study will allow patients access to supervised exercise activities with exercise specialists to help overcome these barriers. With already known general health benefits, the research will feed into larger randomised controlled trials comparing the combination of immunotherapy and pre-defined exercise programmes to standards of care to determine the role exercise can have to improve immunotherapy responsiveness in these cancers. This research aims to improve the physical and mental wellbeing, treatment response and survival outcomes of patients with these poor prognostic cancers.

There is a clear unmet need in both these groups of patients to improve overall response and survival outcomes as well as quality of life. Exercise would be a simple, cost-effective method to utilise in these patients with its known health benefits and potential impact on cancer/treatment related side effects and enhancement of quality of life. This research will further our knowledge on the relationship between exercise and immunity in these advanced cancers. Furthermore, exercise could bridge the gap between the disparity of hot and cold tumour response to immunotherapy.

A13. Please summarise your design and methodology. It should be clear exactly what will happen to the research participant, how many times and in what order. Please complete this section in language comprehensible to the lay person. Do not simply reproduce or refer to the protocol. Further guidance is available in the guidance notes.

Study design

The study will be based in Sheffield. Recruitment will include 30 patients from Weston Park Hospital receiving anti- cancer treatment, either immunotherapy or chemotherapy, for mesothelioma (15 patients) and pancreatic cancer (15 patients). Patients will attend the Advanced Wellbeing Research Centre (AWRC) for a supervised exercise session once a week to include aerobic exercise and resistance training along with an unsupervised weekly exercise session (which the patient will be able to perform at home) for 12 weeks.

We will carry out the following objectives:

1. Feasibility measures include:

1.1) Adherence rate: The ratio of the number of exercise sessions performed to the number of exercise sessions planned.

1.2) Safety of exercise will be assessed by documentation of directly related adverse events.

1.3) Acceptability will be shown by the ratio of those recruited to the study to those approached as eligible.

1.4) The attrition rate will be assessed by the ratio of dropouts from the study to those enrolled initially.

2. Blood samples will be collected at regular time points including at baseline, then 4-weekly pre- and post- exercise interventions for 12 weeks and then at completion of the 12-week exercise intervention.

3. Computed Tomography (CT) imaging at 12 weekly intervals will be performed as standard of care within the NHS to assess evidence that the cancer is getting bigger or smaller.

4. Lung function tests will also be performed at the start and end of the study to assess how well the lungs are working prior to starting the exercise regime as well as at the end of the 12-week exercise regime. This will include spirometry (deep breaths and then blowing hard and fast into a mouthpiece connected tubing).

The following steps outline exactly what will happen for each research participant.
1) Discussion of the study with a member of the research team, including a screening procedure to ensure they fit certain criteria to take part, as well as to ensure there are no unforeseen risks with them carrying out regular exercise activities.
2) Review and sign an informed written consent form
3) Baseline information will be collected including their age, sex, race/ethnicity, medical conditions and medications, normal level of physical activity, type and stage of cancer and information regarding previous cancer treatments.
4) A full medical history including physical examination, baseline measurements of height and weight and general observations will be performed.
5) Blood tests: A blood sample will be taken initially at baseline and then at monthly intervals before and immediately after planned supervised exercise activities. The blood samples will then be analysed to look at changes specifically related to exercise and the potential effect on cancer and cancer treatment. Their blood will not be tested for any other reason without the participant's consent.
6) Exercise activities: The exercise programme will include one weekly supervised exercise session at the Advanced Wellbeing Research Centre based at Olympic Legacy Park, 2 Old Hall Road, Sheffield, S9 3TU and one weekly unsupervised exercise session. The supervised session will include cycling on an exercise bicycle at moderate intensity with periods of rest with an exercise specialist present who will be monitoring their vital signs and tailoring the exercise to ensure the level at which they are exercising is appropriate and safe. The session will also include supervised resistance training. Participants will then be given further advice regarding the unsupervised exercise session from the specialist which will take place at their home. Participants will be given wearable devices (Fitbits) to track their heart rate, monitor activity and sleep. Participants will be advised to wear this during their home exercise and also continuously for 1 week at the beginning of the exercise programme and 1 week at the end. Participants will also have the option to wear the devices all the time. This will allow us to account for physical activity/ exercise outside of the study. These weekly sessions will be arranged so that they do not interfere with any of their cancer appointments or treatments. These sessions will last over a 3 month period.

The following points describe the FITT (Frequency, Intensity, Type, Time) principles that will be used as a plan for the exercise programme.
Frequency: twice weekly, one supervised and one unsupervised session a week for 12 weeks
Intensity: Moderate Intensity aerobic interval exercise (performing at 60% maximum heart rate) combined with 1-3 sets of 6-12 Repetition Maximum (RM) resistance training
Type: Aerobic interval exercise (cycling) and Resistance training (3 exercises: chest press, biceps and leg curl)
Time: at 4 x 4 minute cycling at 60% max HR with 3 minutes active recovery. 3 sets with 2 minutes per set – around 20 minutes resistance training

7) Lung function tests will be performed at baseline and at the end of the study (at 3 months, or earlier if the leave the study sooner). This will assess different capacities in their lungs and how well they are working.
8) Whether they take part in this study or not, if they are receiving anti-cancer treatment for advanced mesothelioma or pancreatic cancer, they will have computed tomography (CT) scans every 3 months. CT scans involve x-ray techniques to assess the disease in the chest, abdomen and pelvis. If they agree to take part in the study, they will have the same number of scans as if they were not in the study.

A14-1. In which aspects of the research process have you actively involved, or will you involve, patients, service users, and/or their carers, or members of the public?

Design of the research
Management of the research

Give details of involvement, or if none please justify the absence of involvement.
We understand the importance of involving patients and their families at all stages in setting up and carrying out this trial. Members of the research team have attended a PPI workshop along with working with experienced members of cancer focus groups to make sure we can listen, make improvements and see whether patients are on board with what we hope to gain from this trial.

This trial has been presented to a dedicated PPI panel at the Mesothelioma UK Research Centre including outlining the feasibility trial, background, aims, methodology and hopeful impact and translational endpoints. We have received very positive feedback and interest regarding this trial from the patients and their families affected by mesothelioma, specifically the concept and that patients would like to be involved in doing something themselves to help their cancer treatment.
PPI has helped shaped the design of this trial with the inclusion of resistance training alongside aerobic exercise within the biweekly exercise sessions as well as to aid on practicalities of improving patient experience in the trial, including allowing family and friends to attend with patient and to ensure appropriate facilities and amenities available for them at the AWRC.

We have presented to a pancreatic cancer focus group via the Pancreatic Cancer UK Research Involvement Network. We outlined the feasibility study, background, aims, methodology and hopeful impact and translational endpoints.
The potential of making immunotherapy beneficial for patients with pancreatic cancer was very well received. The importance of pain control optimisation and early nutritional input were highlighted and key points our clinical researchers will address on the first patient contact.

We will set up a Trial Steering Committee, consisting of members of the research team including collaborators at the Advanced Wellbeing Research Centre and patient and public involvement (PPI) members. This Trial Steering Committee will meet virtually to discuss updates and progress of the study.

The activities the Trial Steering Committee will advise on include supporting grant applications, study recruitment, exercise protocol adherence and any study amendments. The main focus would be on patient safety and satisfaction. Recruitment will be carefully monitored by the Trial Steering Committee and contingency plans considered if recruitment is slower than anticipated.

RISKS AND ETHICAL ISSUES
RESEARCH PARTICIPANTS

A15. What is the sample group or cohort to be studied in this research?
Cancer
Gender: Male and female participants
Lower age limit: 18 years old
Upper age limit: No upper age limit

A17-1. Please list the principal inclusion criteria (list the most important, max 5000 characters).

Patients about to start or who are undergoing first line palliative immunotherapy for advanced, unresectable or metastatic mesothelioma or patients about to start or who are undergoing first line palliative chemotherapy for advanced, unresectable or metastatic pancreatic cancer.
Age over 18 years old
Histological or cytological diagnosis of mesothelioma or pancreatic cancer. ECOG Performance status 0-1 (to be assessed by clinician)
Ability to provide informed consent

A17-2. Please list the principal exclusion criteria (list the most important, max 5000 characters).

Prior treatment with systemic anti-cancer treatment More than one primary cancer

Uncontrolled brain or bone metastases (information will be taken from patient's notes, no additional scans are required at screening to determine this).

Patients who have progressed on first line palliative immunotherapy for advanced, unresectable or metastatic mesothelioma.

Patients who have progressed on first line palliative chemotherapy for advanced, unresectable or metastatic pancreatic cancer.

Patients with active co-morbidities that would prevent or limit their participation in the exercise intervention Age below 18 years old

No histological or cytological diagnosis of mesothelioma or pancreatic cancer. ECOG Performance status of 2 or more

Unable to provide informed consent

A18. Give details of all non-clinical intervention(s) or procedure(s) that will be received by participants as part of the research protocol. These include seeking consent, interviews, non-clinical observations and use of questionnaires.

Please complete the columns for each intervention/procedure as follows:

1. Total number of interventions/procedures to be received by each participant as part of the research protocol.

2. If this intervention/procedure would be routinely given to participants as part of their care outside the research, how many of the total would be routine?

3. Average time taken per intervention/procedure (minutes, hours or days)

4. Details of who will conduct the intervention/procedure, and where it will take place.

Intervention or procedure	1	2	3	4
Seeking informed consent	1	0	30 minutes	Dr Brindley Hapuarachi or person on delegation log within NHS clinics at Weston Park Cancer Centre, Sheffield
International Physical Activity Questionnaire	1	0	30 minutes	Participants will be asked to fill out the questionnaires themselves
Heart rate and step count monitor with wearable device	2	0	12 weeks	Participant will be provided with a wearable device to assess heart rate and step count at specified times during the 12 week exercise programme
Baseline data collection	1	0	15 minutes	Dr Brindley Hapuarachi or person on delegation log within NHS clinics at Weston Park Cancer Centre, Sheffield

A19. Give details of any clinical intervention(s) or procedure(s) to be received by participants as part of the research protocol. These include uses of medicinal products or devices, other medical treatments or assessments, mental health interventions, imaging investigations and taking samples of human biological material. Include procedures which might be received as routine clinical care outside of the research.

Please complete the columns for each intervention/procedure as follows:

1. Total number of interventions/procedures to be received by each participant as part of the research protocol.

2. If this intervention/procedure would be routinely given to participants as part of their care outside the research, how many of the total would be routine?

3. Average time taken per intervention/procedure (minutes, hours or days).

4. Details of who will conduct the intervention/procedure, and where it will take place.

Intervention or procedure	1	2	3	4
First line palliative systemic anti cancer treatment (standard of care immunotherapy or chemotherapy)	4-8	4-8	4-6 hours	Standard of care treatment to be given at Weston Park Cancer Centre by trained chemotherapy nurses

Exercise intervention	24	0	60 minutes	One weekly supervised session at the Advanced Wellbeing Research Centre with an exercise specialist and one weekly unsupervised session for 12 weeks
Blood samples	8	0	10 minutes	Blood samples to be taken before and after exercise at first exercise session and then 4-weekly for the 12-week exercise programme. Bloods will be taken by phlebotomists from the Advanced Wellbeing Research Centre.
Computed Tomography (CT) scans	2	2	30-45 minutes	CT scans to be done as per standard of care treatment at baseline and at 12 weeks to assess for cancer response to treatment on the scans. CT scans will be performed at one of the hospital sites in Sheffield Teaching Hospitals NHS Foundation Trust.
Lung function tests	2	0	120 minutes	Lung function tests including maximal oxygen capacity (VO2 max) and forced expiratory volume (FEV) will be performed at the Advanced Wellbeing Research Centre by trained professionals at the start of the 12-week exercise programme and at the end.
Physical examination including height and weight measurements and vital sign observations (heart rate and oxygen saturations)	1	0	15 minutes	Physical examination including height and weight measurements and vital sign observations (heart rate and oxygen saturations) will be performed by clinicians at Weston Park Hospital at their initial trial visit.

A20. Will you withhold an intervention or procedure, which would normally be considered a part of routine care?

No

A21. How long do you expect each participant to be in the study in total?

13 weeks

A22. What are the potential risks and burdens for research participants and how will you minimise them?

For all studies, describe any potential adverse effects, pain, discomfort, distress, intrusion, inconvenience or changes to lifestyle. Only describe risks or burdens that could occur as a result of participation in the research. Say what steps would be taken to minimise risks and burdens as far as possible.

Risks with exercise intervention - falling / accidents. This risk would be minimised during the supervised session when a trained exercise specialist with experience in working with people with cancer is present to acknowledge individual patient limitations. There will be standardised exercise protocols in place, that can be graded according to the patients' needs and abilities. This will then dictate advice on exercise interventions to be performed unsupervised. The specialist will monitor the patient carefully throughout the session. All exercise protocols have an associated risk assessment that has assessed and mitigated any risks associated with the delivery of physical activity sessions specifically for people living with cancer.

Risks of over-exertion with exercise in a cohort that does not do regular exercise - although exercise is a generally safe intervention, important that patient limitations are addressed and reviewed by our exercise specialists to ensure the participants do not have any cardiovascular, respiratory or musculoskeletal concerns.

Participants will have to attend the Advanced Wellbeing Research Centre weekly for 3 months on top of their standard of care NHS appointments - travel costs will be subsidised as budgeted within the funding.

Possible occurrences

Exercise Intervention

Musculoskeletal injury

Over-exertion: shortness of breath, chest pain.

CT scans: contrast allergy / hypersensitivity.

Long-term effects of radiation including small increase in the potential risk of other cancers.

Lung Function Tests

Dizziness, shortness of breath and cough. Venipuncture

Discomfort, bruising, bleeding, infection around needle site.

A23. Will interviews/ questionnaires or group discussions include topics that might be sensitive, embarrassing or upsetting, or is it possible that criminal or other disclosures requiring action could occur during the study?

No

A24. What is the potential for benefit to research participants?

Evidence suggests that in cancer patients, exercise improves overall health, both physical and mental health and may prolong survival as well as reduce cancer-related fatigue and modulate the immune system through multiple mechanisms.

As well as improving cardiovascular and respiratory functions and so general overall health for the patients, exercise could potentially help with side effects from their anti-cancer treatments as well as hopefully improve the efficacy of their anti-cancer treatments.

A25. What arrangements are being made for continued provision of the intervention for participants, if appropriate, once the research has finished? May apply to any clinical intervention, including a drug, medical device, mental health intervention, complementary therapy, physiotherapy, dietary manipulation, lifestyle change, etc.

As the exercise interventions comprise of one weekly supervised and one weekly unsupervised session, if participant wishes to continue this can be easily done unsupervised and further information can be provided by the advanced wellbeing research centre with regards to further advice.

A26. What are the potential risks for the researchers themselves? (if any)

Nil

RECRUITMENT AND INFORMED CONSENT

In this section we ask you to describe the recruitment procedures for the study. Please give separate details for different study groups where appropriate.

A27-1. How will potential participants, records or samples be identified? Who will carry this out and what resources will be used? For example, identification may involve a disease register, computerised search of GP records, or review of medical records. Indicate whether this will be done by the direct healthcare team or by researchers acting under arrangements with the responsible care organisation(s).

Dr Brindley Hapuarachi (Clinical Research Fellow and PhD student and Specialist Registrar in Medical Oncology) will carry out the process of identifying and recruiting potential participants.

His clinical supervisors for his PhD include Professor Sarah Danson, who is a Consultant Medical Oncologist at Weston Park Cancer Centre and specialises in melanoma, lung and early phase trials, as well as Professor Jonathan Wadlsey, who is a Consultant Clinical Oncologist at Weston Park Cancer Centre and specialises in pancreatic cancer. He will therefore attend new patient mesothelioma and pancreatic cancer and identify patients who fit the inclusion criteria and approach the patients within the clinic to assess interest in the trial.

A27-2. Will the identification of potential participants involve reviewing or screening the identifiable personal information of patients, service users or any other person?

Yes

Please give details below:

The patient's clinical record will be reviewed including laboratory, histopathology and radiological tests on Sheffield ICE clinical system along with referral letters and patients' clinical notes.

A27-3. Describe what measures will be taken to ensure there is no breach of any duty of confidentiality owed to patients, service users or any other person in the process of identifying potential participants. Indicate what steps have been or will be taken to inform patients and service users of the potential use of their records for this purpose. Describe the arrangements to ensure that the wishes of patients and service users regarding access to their records are respected.

Please consult the guidance notes on this topic.

Identifying of potential participants will be done by Dr Brindley Hapuarachi and the clinical supervisors Professor Danson and Professor Wadsley who will directly be involved in patient's standard of care treatments and assessments and so there will be no breach of confidentiality. Dr Patricia Fisher, Dr Robin Young, Dr Fiona Taylor, Professor Matthew Hatton and Dr Tony Mathew are all Consultant Lung Oncologists who treat patients with mesothelioma and will also be approached to be involved in identifying potential participants.

A27-4. Will researchers or individuals other than the direct care team have access to identifiable personal information of any potential participants?

Yes

A27-5. Has prior consent been obtained or will it be obtained for access to identifiable personal information?

Yes

If Yes, please give details below.

Prior written consent will be explicitly obtained within the consenting process for identifiable personal information to be accessed within the research team for research purposes.

A28. Will any participants be recruited by publicity through posters, leaflets, adverts or websites?

No

A29. How and by whom will potential participants first be approached?

Within their standard of care NHS oncology clinics by Dr Brindley Hapuarachi, Professor Sarah Danson or Professor Jonathan Wadsley as well as other Consultant Oncologists and registrars at Weston Park Cancer Centre working within the disease groups mesothelioma or pancreatic cancer.

A30-1. Will you obtain informed consent from or on behalf of research participants?

Yes

If you will be obtaining consent from adult participants, please give details of who will take consent and how it will be done, with details of any steps to provide information (a written information sheet, videos, or interactive material).

Arrangements for adults unable to consent for themselves should be described separately in Part B Section 6, and for children in Part B Section 7.

If you plan to seek informed consent from vulnerable groups, say how you will ensure that consent is voluntary and fully informed.

A written patient information sheet (PIS) will be provided to the patient along with a consultation with chief investigator, Dr Brindley Hapuarachi. He will discuss information relayed within the PIS and explain reasoning behind the study and what it will involve. If patient interested, then contact details will be provided and patient will subsequently sign a consent for the trial at a later consultation.

A30-2. Will you record informed consent (or advice from consultees) in writing?

Yes

A31. How long will you allow potential participants to decide whether or not to take part?

In clinical practice at Weston Park Cancer Centre, patients usually have around 2-4 weeks from new patient consultation to starting first line palliative treatment and therefore this will be the time allowed for potential participants to decide to take part in study or not.

Some patients may wish to consent straight after reviewing/ discussing the PIS, which we will accommodate.

A32. Will you recruit any participants who are involved in current research or have recently been involved in any research prior to recruitment?

Yes

If yes, please give details and justify their inclusion. If not known, what steps will you take to find out?

Participants involved in research would still be eligible if fit the inclusion criteria as would not affect primary or secondary endpoints of the study.

A33-1. What arrangements have been made for persons who might not adequately understand verbal explanations or written information given in English, or who have special communication needs? (e.g. translation, use of interpreters)

Use of translation services / interpreters. A flexible approach will be adapted to ensure the necessary time and appropriate tools are used to ensure persons are well informed and able to make a competent, informed decision.

A34. What arrangements will you make to ensure participants receive any information that becomes available during the course of the research that may be relevant to their continued participation?

Contact details of patients will be obtained, any information that may be relevant to the patient that would require re- consenting will provided to the patient either by post or upon their monthly reviews.

A35. What steps would you take if a participant, who has given informed consent, loses capacity to consent during the study?

The participant would be withdrawn from the study. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to the participant.

A36. Will you be undertaking any of the following activities at any stage (including in the identification of potential participants)?

Access to medical records by those outside the direct healthcare team

Electronic transfer by magnetic or optical media, email or computer networks

Sharing of personal data with other organisations

Use of personal addresses, postcodes, faxes, emails or telephone numbers

Storage of personal data on any of the following:

- Manual files (includes paper or film)
- NHS computers
- University computers
- Laptop computers

Further details:

Data will be shared with research collaborators at the Advanced Wellbeing Research Centre (AWRC), Sheffield Hallam University. Data will be shared via secure University of Sheffield drives with informed consent from participants.

For purposes of making appointments and contacting participants, contact information for participants will be shared with the AWRC via secure google drive via university accounts. Data will be anonymised/pseudo-anonymised for purposes of analysis and shared with University of Sheffield and AWRC when needed via secure google drive via university accounts.

All personnel involved in the study will comply with national guidelines on data protection. All clinical investigators will have direct access to the patient's medical records related to the study. As part of the informed consent process, the patient must be informed that his or her medical records will be reviewed for this purpose.

Data will be collected using study-specific case report forms (CRFs). Designated and trained personnel will collect data at STH and AWRC.

A37. Please describe the physical security arrangements for storage of personal data during the study?

Data will be collected using study-specific case report forms (CRFs). Designated and trained personnel will collect data at STH and AWRC.

Electronic data will be stored in secure, encrypted files on Sheffield Teaching Hospitals NHS devices. Data will be properly archived using an archiving facility. STH has an offsite facility. Any electronic and paper records (site file, written consent forms) will be archived. Identifiable data will be stored in a secure, encrypted place on STH NHS computers only and only to be shared within the research team at STH, University of Sheffield and AWRC with written informed consent from the patient.

All patient data including blood samples and results, clinical information, CT reports and lung function tests will be pseudo-anonymised at Sheffield Teaching Hospitals NHS Foundation Trust (STH) after the patient has consented to the project, but prior to any sample or data transfer. This data will be stored securely only on STH NHS computers and encrypted.

A38. How will you ensure the confidentiality of personal data? Please provide a general statement of the policy and procedures for ensuring confidentiality, e.g. anonymisation or pseudonymisation of data.

Patient data including blood samples and results, clinical information, CT reports and lung function tests will be pseudo-anonymised at Sheffield Teaching Hospitals NHS Foundation Trust (STH) after the patient has consented to the project, but prior to any sample or data transfer. This data will be stored securely only on STH NHS computers and encrypted.

Identifiable data including name and contact details will be shared with the team at AWRC to allow them to organise the exercise appointments. Contact information for participants will be shared with the AWRC via secure google drive via university accounts.

A40. Who will have access to participants' personal data during the study? Where access is by individuals outside the direct care team, please justify and say whether consent will be sought.

The clinical team:

Dr Brindley Hapuarachi Professor Sarah Danson Professor Jonathan Wadsley

Identifiable data including name and contact details will be shared with the team at AWRC to allow them to organise the exercise appointments. Contact information for participants will be shared with the AWRC via secure, encrypted files sent from NHS email using usual NHS mail encryption methods (adding [secure] to email subject).

A41. Where will the data generated by the study be analysed and by whom?

University of Sheffield - Dr Brindley Hapuarachi (Clinical Research Fellow) and University of Sheffield statisticians.

A43. How long will personal data be stored or accessed after the study has ended?

Over 3 years

If longer than 12 months, please justify:

This is a feasibility study to provide further information for a randomised controlled study and therefore, data required for ongoing studies.

A44. For how long will you store research data generated by the study?

5 years

A45. Please give details of the long term arrangements for storage of research data after the study has ended. Say where data will be stored, who will have access and the arrangements to ensure security.

Electronic data to be stored in secure, encrypted file on Sheffield Teaching Hospitals NHS devices.

Data will be properly archived using an archiving facility. STH has an offsite facility. Any electronic and paper records (site file, written consent forms) will be archived.

INCENTIVES AND PAYMENTS

A46. Will research participants receive any payments, reimbursement of expenses or any other benefits or incentives for taking part in this research?

Yes

If Yes, please give details. For monetary payments, indicate how much and on what basis this has been determined. Travel reimbursement - determined within the research team to offer £5 per journey to the Advanced Wellbeing Research Centre, parking is free. Facilities and amenities will be provided for the participants as well as a place for research participants' family or friends to stay during the exercise activity.

A47. Will individual researchers receive any personal payment over and above normal salary, or any other benefits or incentives, for taking part in this research?

No

A48. Does the Chief Investigator or any other investigator/collaborator have any direct personal involvement (e.g. financial, share holding, personal relationship etc.) in the organisations sponsoring or funding the research that may give rise to a possible conflict of interest?

No

NOTIFICATION OF OTHER PROFESSIONALS

A49-1. Will you inform the participants' General Practitioners (and/or any other health or care professional responsible for their care) that they are taking part in the study?

Yes

If Yes, please enclose a copy of the information sheet/letter for the GP/health professional with a version number and date.

A49-2. Will you seek permission from the research participants to inform their GP or other health/ care professional?

Yes

It should be made clear in the participant's information sheet if the GP/health professional will be informed.

PUBLICATION AND DISSEMINATION

A50. Will the research be registered on a public database?

The UK Policy Framework for Health and Social Care Research sets out the principle of making information about research publicly available. Furthermore: Article 19 of the World Medical Association Declaration of Helsinki adopted in 2008 states that "every clinical trial must be registered on a publicly accessible database before recruitment of the first subject"; and the International Committee of Medical Journal Editors (ICMJE) will consider a clinical trial for publication only if it has been registered in an appropriate registry. Please see guidance for more information.

Yes

Please give details, or justify if not registering the research.

We plan to register with clinicaltrials.gov

A51. How do you intend to report and disseminate the results of the study?

Peer reviewed scientific journals

Conference presentation

Publication on website

Results will be disseminated to patient and public involvement groups including Mesothelioma UK. We will keep all

those involved updated on the status of the study and where we hope to publish the information. We will also contact Mesothelioma UK and Pancreatic Cancer UK to allow this research to be available freely on their websites for the public.

A52. If you will be using identifiable personal data, how will you ensure that anonymity will be maintained when publishing the results?

No patient identifiable information will be present within any of the published data

A53. How and when will you inform participants of the study results?

If there will be no arrangements in place to inform participants please justify this.

We will ask all study participants if they want to be told the study results and if so, we will send them a study summary.

Scientific and Statistical Review

A54. How has the scientific quality of the research been assessed?

Review within the Chief Investigator's institution or host organisation

Review within the research team

Review by educational supervisor

Justify and describe the review process and outcome. If the review has been undertaken but not seen by the researcher, give details of the body which has undertaken the review:

It has been reviewed in line with STH's Independent Scientific Review SOP.

As part of this process, the research will be reviewed internally within the Department of Oncology and Metabolism, University of Sheffield.

A57. What is the primary outcome measure for the study?

Adherence rate: The ratio of the number of exercise sessions performed to the number of exercise sessions planned.

A58. What are the secondary outcome measures?(if any)

i. The number of directly related adverse events.

ii. Acceptability rate: the ratio of those recruited to the trial to those approached as eligible.

iii. Attrition rate: the ratio of dropouts from the trial to those enrolled initially.

iv. Peripheral blood mononuclear cells (PBMC) in the blood at defined timepoints . Immune cell activation will be determined by an increased in cytotoxic immune components (Natural Killer cells / CD8 T cells) and a decrease in immunosuppressive immune cells (regulatory T cells).

v. The levels of cytokines/ myokines in the blood at defined timepoints.

vi. Evidence of radiological response or progression as per RECIST 1.1 reporting of CT scans at defined timepoints.

vii. Lung function as per results of lung function tests at defined timepoints.

A59. What is the sample size for the research? How many participants/samples/data records do you plan to study in total? If there is more than one group, please give further details below.

Total UK sample size: 30

Total international sample size (including UK): 30

Further details:

15 patients with mesothelioma 15 patients with pancreatic cancer

A60. How was the sample size decided upon? If a formal sample size calculation was used, indicate how this was done, giving sufficient information to justify and reproduce the calculation.

This is a feasibility study of exercise regimens in two different cancer groups and so power calcultations are not applicable. After discussion with Dr Munitta Muthana, Professor Sarah Danson and Professor Jonathan Wadsley, the sample size chosen was felt adequate to gain feasibility data as well as discussion with mesothelioma and pancreatic cancer Oncologists to discuss the practicalities of recruiting the sample size over 12 months.

We have discussed with Nikki Totton (Medical Statistician at the NIHR Research Design Service (RDS) Yorkshire and Humber) who has confirmed the acceptability of the sample size chosen given practicalities of recruiting and adequacy in collecting feasibility data given the possible variability in the measures including compliance rate.

A61. Will participants be allocated to groups at random?

No

A62. Please describe the methods of analysis (statistical or other appropriate methods, e.g. for qualitative research) by which the data will be evaluated to meet the study objectives.

Feasibility of exercise dictated by the adherence rate:

The ratio of the number of exercise sessions performed to the number of exercise sessions planned. Safety of exercise – directly related adverse events

Acceptability of exercise dictated by recruitment:

The ratio of those recruited to the study to those approached as eligible The attrition

The ratio of dropouts from the study to those enrolled initially

Quantitative data:

Non-parametric tests to assess whether there are statistically significant differences between the biological/immune components of blood pre and post exercise.

A74. What arrangements are in place for monitoring and auditing the conduct of the research?

Standard operating procedures at University of Sheffield and Sheffield Teaching Hospitals NHS Foundation Trust.

Sheffield Teaching Hospitals NHS Foundation Trust has a responsibility to ensure that all research within the Trust is conducted in accordance with the relevant legislation and guidelines. In order to assist research teams in fulfilling their obligations, the Clinical Research and Innovation Office has developed a suite of SOPs covering key activities. These SOPs will be included in the study Site File.

A75-1. What arrangements will be made to review interim safety and efficacy data from the trial? Will a formal data monitoring committee or equivalent body be convened?

As a feasibility study of an exercise programme, safety is one of the primary feasibility outcome measures. This will be continually assessed under the exercise specialists at the Advanced Wellbeing Research Centre. Further to this, arrangements will be made for a regulatory inspection as a standard operating procedure from the Clinical Research and Innovation Office.

A75-2. What are the criteria for electively stopping the trial or other research prematurely?

Failure to recruit

Safety concerns with exercise intervention

A76-1. What arrangements will be made for insurance and/or indemnity to meet the potential legal liability of the sponsor(s) for harm to participants arising from the management of the research? Please tick box(es) as applicable.

Note: Where a NHS organisation has agreed to act as sponsor or co-sponsor, indemnity is provided through NHS schemes. Indicate if this applies (there is no need to provide documentary evidence). For all other sponsors, please describe the arrangements and provide evidence.

NHS indemnity scheme will apply (NHS sponsors only)

A76-2. What arrangements will be made for insurance and/ or indemnity to meet the potential legal liability of the sponsor(s) or employer(s) for harm to participants arising from the design of the research? Please tick box(es) as applicable.

Note: Where researchers with substantive NHS employment contracts have designed the research, indemnity is provided through NHS schemes. Indicate if this applies (there is no need to provide documentary evidence). For other protocol authors (e.g. company employees, university members), please describe the arrangements and provide evidence.

NHS indemnity scheme will apply (protocol authors with NHS contracts only)

Other insurance or indemnity arrangements will apply (give details below)

The University of Sheffield (UoS) is the substantive employer for some of the research designers. The UoS is a policy holder with Zurich Insurance Company Ltd. Policy number is NHE-03CA06-0013

PART B: Section 3 – Exposure to ionising radiation

1. Does the study involve exposure to radioactive materials?

No

2. Does the study involve other diagnostic or therapeutic ionising radiation?

Yes

B1. Details of other ionising radiation

Procedure	Number of procedures	Estimated procedure dose
CT Thorax, abdomen and pelvis with contrast	2	19 mSv (Br J Radiol 2016; 89:20150346

C. Dose and risk assessment

C1. What is the total participant dose from all the exposures in A1 and/or B1, and what component of this is the additional dose over and above standard practice? What are the risks associated with these two doses (total and additional)?

The dose and risk assessment should be set out below. This should be prepared by a Medical Physics Expert (MPE) who is a registered clinical scientist registered with the Health Professions Council and has expertise relevant to the planned exposures. Where the study involves different types of exposure (for example, both radioactive materials and other ionising radiation, or more than one imaging method), advice may need to be sought from other MPEs with relevant expertise. The lead MPE should produce a combined assessment for the ethics committee, giving the names of any other MPEs who have contributed to the assessment. Further guidance is available by clicking on the information button.

MPE report

This study requires exposures to ionising radiation which are detailed in A1 and B1. All of the exposures required by the study are normal routine clinical care. The total protocol dose is 38 mSv. This is equivalent to 16.5 years of average natural background radiation in the UK.

Ionising radiation can cause cancer which manifests itself after many years or decades. The risk of developing cancer as a consequence of taking part in this study is 0.19 %, which is low. For comparison, the natural lifetime cancer incidence in the general population is about 50%.

The following risk statement needs to be included in the PIS:

The CT scans you will receive are part of your routine care. If you take part in this study you will not undergo any additional procedures. These procedures use ionising radiation to form images of your body and provide your doctor with other clinical information. Ionising radiation can cause cell damage that may, after many years or decades, turn cancerous. The chances of this happening to you are the same whether you take part in this study or not.

C2. Declaration by lead Medical Physics Expert

I am satisfied that the information in sub-sections A and/or B and the assessment in sub-section C provide a reasonable estimate of the ionising radiation exposure planned in this research and the associated risks.

This section was signed electronically by Mr Giles Morrison on 15/03/2024 11:40.

D1. Will the exposure exceed the exposure that might be received as part of normal care at any proposed research site?

Yes

D2. Assessment of additional exposure

Explain how the planned exposure compares with normal practice and assess whether it is appropriate, using language comprehensible to a lay person. Consideration should be given to the specific objectives of the exposure, the characteristics of participants, the potential diagnostic or therapeutic benefits to the participant, the potential benefits to society, the risk to the participant and the availability of alternative techniques involving less, or no, ionising radiation.

If pregnant or breast-feeding mothers are to be studied give reasons and details of special radiation protection measures to be taken.

CRE report

Study design:

Determine the feasibility and acceptability of exercise during immunotherapy treatment in patients with mesothelioma as well as in a non-immunotherapy treated cohort of patients with pancreatic cancer.

15 patients with advanced mesothelioma (on or due to start first line palliative nivolumab and ipilumumab) – median overall survival 18.1 months

15 patients with advanced pancreatic cancer (on or due to start first line palliative chemotherapy) – median overall survival 11.1 months

Objectives (relevant to imaging/radiation exposure):

a) Computed Tomography (CT) imaging at regular time points will be performed to assess evidence of radiological response or progression as per RECIST 1.1.

b) Prepare a clinical trial protocol: Combining immunotherapy with exercise to improve the responsiveness to immunotherapy in a larger cohort.

Imaging Procedure requirements:

CE CT CAP at baseline and at 3 months, reported according to RECIST 1.1

The exposures are in line with standard clinical care in terms of frequency a participant will not receive additional exposure as a result of participation.

Additional reporting requirements – RECIST 1.1 is above standard clinical care and will require 3d lab input/funding.

Summary:

The 2 CE CT CAP exposures are part of standard clinical care not additional, both exposures are justifiable on that basis.

D3. Declaration by lead Clinical Radiation Expert

I am satisfied that the exposure to ionising radiation planned in this research study (as defined in A1 and/or B1) is reasonable and that the risks are adequately described in the participant information sheet for the study.

This section was signed electronically by Dr Eleanor Lorenz on 19/03/2024 10:01.

Part B: Section 5 – Use of newly obtained human tissue (or other human biological materials) for research purposes

1. What types of human tissue or other biological material will be included in the study?

Blood samples

2. Who will collect the samples?

Phlebotomist at the Advanced Wellbeing Research Centre as well as phleobotomists at Weston Park Cancer Centre

3. Who will the samples be removed from?

Living donors

4. Will informed consent be obtained from living donors for use of the samples? Please tick as appropriate

In this research?

Yes

In future research?

Yes

Will any tissues or cells be used for human application or to carry out testing for human application in this research?

No

9. What types of test or analysis will be carried out on the samples?

Blood samples taken and biological measures assessed including peripheral blood mononuclear cell (PBMC) characterisation, inflammatory, metabolic, oxidative stress biomarkers, and molecular profiling. We will use a combination of Flow cytometry, cytokine bead-based multiplex immunoassays as well as RNA-seq to fully profile changes in gene and protein expression.

These will be extra blood samples (not standard of care). This will be taken before and after exercise session at the AWRC (at the initial exercise session then at 4-weekly intervals until the end of the 12-week exercise intervention).

13. Give details of where the samples will be stored, who will have access and the custodial arrangements.

Samples will be stored at the University of Sheffield.

14. What will happen to the samples at the end of the research?

Transfer to research tissue bank

Disposal in accordance with the Human Tissue Authority's Code of Practice

Please give further details of the proposed arrangements:

Samples will be stored in the Biorepository at University of Sheffield.

The University of Sheffield Medical School holds a licence for the storage of human tissue for the purposes of research. Before 2019 the university and Sheffield Teaching Hospitals Trust held a joint licence creating the Biorepository to satisfy all requirements under the regulations. Professor of Clinical Cardiology and Honorary Consultant Cardiologist, Professor Chris Newman, holds the licence as the designated individual responsible for the Sheffield Biorepository; copies of this licence can be viewed in room EU17 of the Medical School.

Appendix B:

Yorkshire Cancer Research (YCR) More Life to Live (MLTL) application

Research Award 2024 Round 2

RA\2024\R2\109

Exercise to Boost Immunity in advanced Cancer: feasibility of combined aerobic Exercise and resistance training for Patients with advanced mesothelioma and pancreatic cancer (BICEP)

Background

Immunotherapy is a cancer treatment that helps your own immune system fight cancer. Immunotherapy is standard of care in advanced mesothelioma patients, however better and longer responses are needed. Immunotherapy is not currently beneficial for patients with advanced pancreatic cancer and prognosis remains poor.

Exercise can improve the physical and mental wellbeing of cancer patients. Exercise can also boost the immune system and potentially make immunotherapy work better and improve survival outcomes in patients with cancer.

What we aim to do

Firstly, we need to find standard exercise activities that advanced cancer patients find acceptable and can continue long term. We will also look into how exercise affects the immune system and lung function in these patients.

We will then design a larger study looking into the effect of exercise on how well immunotherapy works and whether this improves survival.

Study recruitment and design

We will recruit patients with advanced mesothelioma and pancreatic cancer who are on or starting immunotherapy or chemotherapy.

The exercise programme will include one supervised and one unsupervised session per week and involve cycling and strength training supervised by a specialist and tailored to the individual. One session will last around 30-45 minutes and take place over 3 months.

We will take blood samples monthly, before and after exercise and assess the response of the cancer to treatment using medical imaging. Importantly, we will hear directly from patients about whether they feel exercise has affected their physical and mental health using questionnaires during the study.

Project Title
Exercise to Boost Immunity in advanced Cancer: feasibility of combined aerobic Exercise and resistance training for Patients with advanced mesothelioma and pancreatic cancer (BICEP)

Total Support Requested
£118,754.00

Start date
01 February 2025

End date
01 April 2026

Project Length
15

Priority Area
Improving treatments

Project Need (200 words max)
Pancreatic cancer is the 10th most common UK cancer and the 5th most common cause of cancer-related death, with a 5 year survival rate of less than 7%. Mesothelioma accounts for less than 1% of all new UK cancer cases however incidence has increased by 57% over the past 30 years, due to occupational asbestos exposure. Immunotherapy is used as standard of care in patients with advanced mesothelioma, but only has small benefits and does not work in pancreatic cancer. There is a clear unmet need in both these patients to improve immunotherapy response and survival outcomes. Exercise would be a simple, cost-effective method to use in these patients with its known health benefits and potential to boost immunity and make immunotherapy work better.

There is currently no tailored exercise advice for patients with advanced cancer. This research is vital to establish acceptable exercise regimes for patients with advanced mesothelioma and pancreatic cancer. This feasibility study will aim to define this exercise and demonstrate that exercise boosts immunity in patients with difficult-to-treat advanced cancers. This will allow larger studies exploring how effective the combination of exercise and immunotherapy is in these patients and if this improves the treatment and survival.

Technical Summary (200 words max)
Background

Immune checkpoint inhibitors (ICIs) are not effective against pancreatic cancer; a 'cold' tumour lacking effector immune cells within its microenvironment. Mesothelioma lies in the middle of the cold-hot spectrum, with modest responses to ICIs but vast room for improvement. Moderate-intensity exercise can turn cold tumours hot by increasing Natural Killer and cytotoxic T-cells, diminishing immunosuppressive myeloid-derived suppressor cells and normalising tumour vasculature reducing hypoxia. Mouse studies show exercise and immunotherapy together is effective in pancreatic cancer. This trial will determine the feasibility of individually tailored moderate-intensity aerobic exercise and resistance training during treatment in patients with advanced pancreatic cancer and mesothelioma.

Methods
Recruitment of thirty patients receiving first-line immunotherapy or chemotherapy for advanced mesothelioma and pancreatic cancer. Patients will undertake a weekly supervised and unsupervised exercise session, including aerobic and resistance training, for 12 weeks. Cytokine and immune cell concentration will be analysed from blood samples collected pre and post supervised sessions. Standard-of-care CT scans will determine disease response. Lung function tests will assess changes in forced expiratory volume and maximal oxygen capacity.

Analysis

Feasibility measures including adherence, acceptability and attrition rates will be determined along with safety. Non-parametric analyses will determine any statistically significant differences in quantitative data.

Section 3 - Project Impact and Benefit
Study design
Please provide a brief summary of your study design (250 words max)
Exercise for both of the two-arm feasibility study (15 pancreatic cancer and 15 mesothelioma patients):

Frequency: Twice weekly (one supervised and one unsupervised, wearable device (Fitbit) provided) for 12 weeks.
Intensity: Moderate interval exercise (build up to 60% maximum heart rate depending on individual capabilities). 1-3 sets of 6-12 Repetition Maximum resistance training tailored to individual capabilities.
Type: Aerobic (cycling) and Resistance training (chest press, biceps and leg curl).
Time: 4x4-minute cycling with 3-minute recovery. 3x2-minute resistance training sets. Intensity/duration of exercise will be adjusted to individual capabilities.

Investigations
Lung function tests (forced expiratory volume and maximal oxygen capacity) at baseline and study completion.
Standard-of-care CT scans done 3-monthly (maximum 2 scans/participant). No further scans required for study. Disease response will be assessed using RECIST 1.1 criteria.
Blood samples at baseline and 4-weekly for 12 weeks, pre and post supervised exercise sessions at the AWRC. Cytokine profiles, Natural Killer cells, CD4 T- cells, regulatory T-cells, CD8 T-cells, myeloid/myeloid-derived suppressor cells and T-cell activation markers will be analysed using flow cytometry, RNA extraction/PCR and NanoString immune profiling at the University of Sheffield.
Quality of life assessed using EQ-5D questionnaires.

Safety:
Risk of an accident during exercise will be minimised where an exercise specialist with experience working with people with cancer is present acknowledging individual limitations and tailoring exercise to the individual.
Standardised risk-assessed exercise protocols will mitigate risks specifically for people with cancer and can be graded to the patients' needs and abilities dictating advice on unsupervised exercise.

Number of people in the control/ receiving standard care only within the study
0

Number of people in the experimental group(s) within the study
30

Breakdown by experimental group
30 patients receiving immunotherapy for mesothelioma (n=15) or chemotherapy for pancreatic cancer (n=15) will be recruited from South Yorkshire over 12 months. The sample size was chosen under advice from a medical statistician accounting for variability around feasibility measures, recruitment practicalities and adequacy to determine exercise feasibility.

An estimated 250 patients/year present with pancreatic cancer in South Yorkshire (population 1.4 million). From Pancreatic Cancer UK statistics (1),
70% of patients deemed too unwell for any intervention and 10% of patients presenting early enough for curative surgery, recruiting 15 patients (6% of new pancreatic cancer patients in South Yorkshire) was agreed to be acceptable and practical.

With an incidence of 2718 new cases of mesothelioma per year in the UK, an estimated 60/year present with mesothelioma in South Yorkshire. On consultation with Consultant Medical Oncologists specialising in Mesothelioma at Weston Park Cancer Centre, Sheffield, recruiting 15 patients over 12 months who are about to start or who are currently on immunotherapy was deemed to be realistic and acceptable.

(1) Pancreatic cancer statistics - Pancreatic Cancer UK [Internet]. [cited 2024 Mar 8]. Available from: https://www.pancreaticcancer.org.uk/what-we-do/media- centre/pancreatic-cancer-statistics/

In the experimental group(s), what is the expected adherence rate to the intervention(s) within your study? (100 words max)
Pancreatic cancer arm:
Adherence rate to exercise interventions is expected to be 50%. This would account for patients unable to perform activities due to treatment toxicities, disease progression and death.

Mesothelioma arm:
Adherence rate to exercise interventions is expected to be 60%. A higher adherence rate is expected within the mesothelioma arm due to reduced frequency of severe toxicities, better cancer prognosis than advanced pancreatic cancer and previous feasibility studies looking into advanced lung cancer and exercise (Olivier et al, 2018).

Number of people that could receive the intervention if rolled out across Yorkshire
400

Please summarise any evidence and assumptions used to calculate the numbers reported above. (250 words max)
Please note, this is 400 people per annum.

With an incidence of 15.4/100,000 for women and 20.2/100,000 for men, an estimated 960 patients per year are expected to present with pancreatic cancer in Yorkshire (population of Yorkshire is 5.4 million (1,2)). With up to 70% of patients deemed too unwell for any intervention and 10% of patients presenting early enough for curative surgery, around 200 (approximately 20%) new patients with pancreatic cancer could receive the exercise intervention if rolled out across Yorkshire (2).

With an incidence of 2718 new cases each year, an estimated 220 patients per year are expected to present with mesothelioma in Yorkshire (3). 15-20% of patients may be eligible for surgery, although in practice this is likely lower, meaning around 200 new patients with mesothelioma could receive the exercise intervention (4).

400 people with new diagnoses of either pancreatic cancer or mesothelioma may therefore be able to receive the intervention per annum. Numbers may be greater than this if rolled out to patients already established on treatment.

Population of Yorkshire 2023 - UK Population Data [Internet]. [cited 2024 Feb 1]. Available from: https://populationdata.org.uk/population-of-yorkshire/
Pancreatic cancer statistics - Pancreatic Cancer UK [Internet]. [cited 2024 Feb 1]. Available from: https://www.pancreaticcancer.org.uk/what-we-do/media- centre/pancreatic-cancer-statistics/
Mesothelioma statistics | Cancer Research UK [Internet]. [cited 2024 Feb 1]. Available from: https://www.cancerresearchuk.org/health-professional/cancer- statistics/statistics-by-cancer-type/mesothelioma#heading-Two.
2023 Mesothelioma Report: Latest Mesothelioma Survey [Internet]. [cited 2024 Feb 1]. Available from: https://www.asbestos.com/featured-stories/state-of- mesothelioma-2023/#:~:text=According to the survey%2C 18%25 of mesothelioma patients, for older patients or those with chronic illnesses.

Intervention specific impact and outputs

Is your study focused on any of the following interventions?

<u>**Exercise**</u>
Description of population eligible for this exercise intervention. (150 words max)

Population eligible for this exercise intervention in this study will include patients from Weston Park Cancer Centre, Sheffield covering the population across South Yorkshire. Patients will be about to start or currently receiving first line palliative immunotherapy for advanced, unresectable or metastatic mesothelioma (n=15) and patients about to start or currently receiving first line chemotherapy for advanced pancreatic cancer (n=15).

When will the exercise intervention take place? During treatment

Please describe in more detail when this exercise intervention will be offered. (150 words max)

Exercise intervention will be offered to any patient who is either about to start or who has already started and not progressed on first-line palliative chemotherapy for advanced pancreatic cancer or palliative immunotherapy for advanced mesothelioma. First-line palliative chemotherapy in pancreatic cancer is usually given over 24 weeks and palliative immunotherapy in mesothelioma can be given for up to 2 years. Therefore, patients could be eligible if have 12 or more weeks left of treatment.

Patients would need to be eligible for these treatments and therefore have a performance status of 0-1 (from fully active to ambulatory but restricted with more physically strenuous activity). Exercise interventions will occur during their treatment.

	During the study?	If programme rolled out across Yorkshire?

Additional number of referrals to an exercise programme as a result of this study.	30	400
Additional number of people taking part in an exercise programme as a result of this study.	30	400
Additional number of people completing an exercise programme as a result of this study.	15	200

Please briefly describe how your study defines 'taking part' and 'completion' of an exercise programme. (150 words max)

Taking part would be defined as attending at least one of the planned supervised exercise sessions. Completion of the exercise programme would be performing 50% or more of the planned supervised and unsupervised exercise sessions over 12 weeks.

Please summarise any evidence and assumptions used to calculate the numbers reported above. (250 words max)

Mikkelsen et al. showed that strength training in combination with a home-based walking programme over 12 weeks was feasible in an elderly population affected by either advanced pancreatic cancer, biliary tract cancers or non- small cell lung cancer (1). They demonstrated a 69% adherence rate and 94% completion rate of attended sessions occurring alongside first line palliative oncological treatment (1). The acceptability of this exercise intervention by an older age population and the inclusion of hard-to- treat advanced cold tumours is promising for the future incorporation of exercise into clinical practice in these populations. However, it is important to note the exercise intervention was set at a lower intensity than this study, so adherence is expected to be lower (50%).

A combination of cycling and resistance training was incorporated into a rehabilitation programme for patients with advanced lung cancers including mesothelioma (2). There was a 56.6% completion rate of the 8-week programme
(2). This supports an expected 50% adherence rate for this study.

Mikkelsen MK, Lund CM, Vinther A, Tolver A, Johansen JS, Chen I, et al. Effects of a 12-Week Multimodal Exercise Intervention Among Older Patients with Advanced Cancer: Results from a Randomized Controlled Trial. Oncologist [Internet]. 2022 Jan 1 [cited 2023 Jul 5];27(1):67. Available from: /pmc/articles/PMC8842365/

Olivier C, Grosbois JM, Cortot AB, Peres S, Heron C, Delourme J, et al. Real-life feasibility of home-based pulmonary rehabilitation in chemotherapy-treated patients with thoracic cancers: a pilot study. BMC Cancer [Internet]. 2018 Feb 13 [cited 2023 Jul 5];18(1). Available from: /pmc/articles/PMC5810120/

Further information – exercise. (250 words max)

To assess the promotion of anti-tumour immunity by exercise in advanced mesothelioma and pancreatic cancer, blood samples will be collected at baseline and 4-weekly for 12 weeks, pre and post the supervised exercise session at the AWRC. Th1 and Th2 cytokines and leucocyte subpopulations (Natural Killer cells, CD4 T-cells (including regulatory T-cells), CD8 T-cells, CD20 B-cells, CD14 monocytes and activation markers CD25, CD69 and HLA-DR) will be analysed using flow cytometry, RNA extraction/PCR and NanoString immune profiling at the University of Sheffield.

CT scans done every 3 months as standard-of-care whilst undertaking the exercise interventions will be reported to RECIST 1.1 criteria. This will assess the radiological response of the combination of exercise and treatment on the advanced cancers. This can be compared to established data on responses to standard-of-care treatments .

Blood samples and CT scans analysis will then feed into a larger, robust randomised controlled trial assessing combination of exercise and immunotherapy in these cancers to improve treatment responses and survival outcomes in these poor prognostic cancers.

Lung function tests will be performed at baseline and study completion. Differences in forced expiratory volume and maximal oxygen capacity will be calculated to assess if respiratory function improves in these advanced cancers, where reduced lung function can impair their tolerability of treatment. This may indicate exercise can improve tolerance, adherence and completion of exercise.

Quality of life will be assessed.

Early diagnosis of cancer

Do you expect this study will lead to more cancers being diagnosed at an earlier stage? No

Improved treatment

Is your proposal related to the treatment of cancer? Yes

Please describe the main ways in which your study will improve treatment. (250 words max)

Exercise can benefit patients with cancer by improving overall survival, reducing cancer-related and treatment induced fatigue and reducing cancer recurrence. Growing evidence indicates exercise can modulate local tumour and systemic immunity in patients with cancer, favouring anti-tumour activity and supporting immunotherapy's mechanisms of action. Studies suggest moderate intensity, aerobic exercise and resistance training over 8- 12 weeks can enhance anti-tumour immunity. Therefore, this project could show exercise is crucial in improving responses and survival in patients undergoing immunotherapy, such as advanced mesothelioma and expanding the spectrum of cancers that can benefit from immunotherapy, such as pancreatic cancer.

This research is vital to establish acceptable exercise regimes for patients with advanced mesothelioma and pancreatic cancer who can be significantly limited by respiratory difficulties, cancer cachexia and frailty. This study will allow patients access to supervised exercise activities with exercise specialists to help overcome these barriers. With already known general health benefits, the research will feed into a larger randomised controlled trial comparing the combination of immunotherapy and exercise to immunotherapy alone in mesothelioma.

Due to lack of efficacy, immunotherapy alone cannot be compared to an exercise – immunotherapy combination in cold tumours. Therefore, a randomised controlled trial comparing the immunotherapy-exercise combination against exercise alone and surveillance alone (standard of care) in advanced pancreatic cancer, will be assessed as a maintenance strategy in patients that have completed first line palliative chemotherapy and have either responded or have stable disease. Weston Park Hospital is consistently a top recruiter for pancreatic cancer trials.

With an incidence of 15.4/100,000 for women and 20.2/100,000 for men, an estimated 960 patients per year are expected to present with pancreatic cancer in Yorkshire (population of Yorkshire is 5.4 million (1,2)). With up to 70% of patients deemed too unwell for any intervention and 10% of patients presenting early enough for curative surgery, around 200 (approximately 20%) new patients with pancreatic cancer could receive the exercise intervention if rolled out across Yorkshire (2).

With an incidence of 2718 new cases each year, an estimated 220 patients per year are expected to present with mesothelioma in Yorkshire (3). 15- 20% of patients may be eligible for surgery, although in practice this is likely lower, meaning around 200 new patients with mesothelioma could receive the exercise intervention (4).

Further information – treatment. (250 words max)
Exercise has been shown to have health benefits, both physically by improving cardiovascular, respiratory and musculoskeletal functioning and mentally, reducing levels of anxiety and depression. Specifically, exercise has been shown to help reduce fatigue associated with cancer as well as its treatment. This study will provide free access, for patients with these advanced cancers, to supervised exercise along with the resources and equipment to perform these exercise activities. Therefore patients could benefit from the general positive impact on physical and mental health. This in turn could reduce treatment toxicity, improve treatment tolerance and adherence and therefore promote maintenance of anti-cancer treatment dose intensity to ensure best treatment responses.

Furthermore, treatment for advanced pancreatic cancer includes either platinum- or taxane-based chemotherapy which can cause peripheral neuropathy, which can be dose limiting and persistent. Moderate intensity exercise has been shown to reduce neuropathic symptoms such as peripheral hot/coldness, numbness and tingling. This again may help improve treatment tolerance and maintain dose intensity to maximise treatment benefit.

Please summarise any evidence and assumptions used to calculate the numbers reported above. (250 words max)
In a 2019 systematic review of 25, 563 breast cancer patients, exercise was shown to lower the risk of breast cancer related death by 40%. https://link.springer.com/article/10.1007/s10549-017-4541-9

Impact
Please detail any other significant patient benefit / impact not already described? (250 words max)

South Yorkshire has areas affected by higher relative risk of death by mesothelioma than other sites in the UK, including Doncaster (relative risk of death by mesothelioma 1.611). Therefore, this project aims to directly reduce this relative risk by improving existing treatment (immunotherapy) with addition of exercise and subsequently, improving survival outcomes.

The prognosis of metastatic pancreatic cancer remains poor with a median overall survival of 11.1 months with optimal chemotherapy and a 30% response rate. Only 40% of patients with mesothelioma having immunotherapy will respond with a median overall survival of 18.1 months.

Exercise would be a simple, cost-effective method to utilise in these patients with known health benefits and potential impact on side effects and quality of life enhancement. This project will establish acceptable, safe regimes in patients with these advanced cancers and plan to demonstrate an enhanced immune response to exercise in these patients, supporting its use as an adjunct to immunotherapy.

This will allow a randomised controlled trial assessing the combination of exercise and immunotherapy against immunotherapy monotherapy or exercise alone. Progression free survival would be primary endpoint along with secondary endpoints of overall survival and objective response rate on imaging. This feasibility study is crucial to define the 'exercise dose' required to feed into a randomised controlled trial to demonstrate improved survival with a combination of immunotherapy and exercise in patients with these advanced cancers. Subsequently, clinicians will be able to prescribe a tailored dose of exercise alongside immunotherapy to improve responses and survival.

Health inequalities
All applicants should select 'Yes' to ONE of the following questions only. Is the primary focus of your study related to Funding Round Topics 1 to 3?
Yes

Please describe whether your study will reduce cancer-related health inequalities in Yorkshire. (250 words max)

Patients across Yorkshire will have varying access to exercise facilities and resources. Many patients may not have an opportunity to gain input and supervision from exercise specialists due to multiple reasons including socioeconomic differences and language barriers. This study will provide this expertise and supervision to many
patients who may otherwise not be able to gain the benefits of exercise as they either don't have the means to do so or don't feel safe to exercise alone.

This study will define a safe exercise regime that patients with advanced mesothelioma and pancreatic cancer can perform during treatment and in the long term. This includes activities within an exercise facility and also home-based exercises performed with Fitbits. Therefore, this project will offer invaluable advise regarding what exercise patients with advanced cancer can do at home on their own, providing health benefits to patients that may not be able to access exercise facilities and reducing cancer-related health inequalities.

Furthermore, this study will build on evidence to support a randomised controlled trial to confirm whether the combination of immunotherapy and exercise provides the best responses and survival outcomes. With this information, exercise should be treated as an important intervention to improve treatment responses and survival outcomes in patients with mesothelioma and pancreatic cancer and therefore, warrant NHS funding. The Advanced Wellbeing Research Centre has the expertise, facilities and resources to help deliver these interventions as well as involve commercial exercise facilities to provide NHS-funded exercise classes, reducing health inequalities across different socio-economic groups.

Please describe the steps you will take to ensure the research population is representative of the Yorkshire population for the condition under investigation. (300 words max) You should carefully consider any extra measures needed to recruit participants from groups which are typically underrepresented in research.
All eligible patients that attend mesothelioma and pancreatic cancer clinics at Weston Park Hospital, covering the population across South Yorkshire, over the duration of recruitment will be approached to discuss the study. As all patients with advanced pancreatic cancer undergoing palliative systemic treatment in South Yorkshire will be seen and treated at Weston Park Hospital, we will be recruiting from the representative population. Most patients with advanced mesothelioma will be seen by clinicians from Weston Park Hospital. Therefore patients from different geographical and socioeconomic groups will be approached across South Yorkshire.

Where needed, interpreters will be used to ensure there are no issues with language barrier at all steps within the study. Participants will also be able to bring their family / friends to the AWRC for each exercise activity. The study intervention involves travel to the Advanced Wellbeing Research Centre (AWRC). Transport costs to and from the AWRC will be covered. Snacks, drinks and refreshments will also be provided. Therefore, there will be no financial burden on participants, and they will be able to receive the benefits of supervised tailored
exercise regime at no cost. The populations affected by these cancers will be at a more advanced age and therefore careful consideration of transport, supervision and tailoring of any exercise activity to the individual will take place and this will all be emphasised to patients during recruitment.

In all our cancer research, we strive for equality in our patient recruitment to ensure that we represent the Yorkshire population. In order to achieve inclusion, we have already established connections to community groups including under represented groups and religious organisations.

Please summarise any evidence and assumptions used to work out the impact on health inequalities in Yorkshire. (250 words max)

According to an Active Lives Adult Survey from Sport England, with the introduction of lockdowns and shielding, more than 3 million people were less active (1). It was highlighted that access to facilities for specific groups including people aged 70 and over, people with long-term health conditions and people from Black, Asian and other minority ethnic groups were disproportionately impacted with regards to this. The advanced mesothelioma and pancreatic cancer populations within the study will comprise mainly of the first two groups mentioned previously. Furthermore, with the use of interpreting services and the ability for study participants to bring family and friends to the exercise facility for support/ interpretation, this study will tackle these issues these groups may face with regards to accessing exercise facilities.

In Yorkshire and Humber around 1 in 5 residents live in geographical areas that are classified as being in the most deprived decile of England. This highlights the need for both NHS-funded exercise activities and a defined dose of exercise that can be performed at home without financial burden on patients. This project is vital in providing the evidence for a large randomised controlled trial to explore benefits of an immunotherapy-exercise combination.

1. Activity habits in early weeks of lockdown revealed | Sport England [Internet]. [cited 2024 Feb 9]. Available from: https://www.sportengland.org/news/activity- habits-early-weeks-lockdown-revealed

Further information – health inequalities. (250 words max)

The Advanced Wellbeing Research Centre (AWRC) is located in Darnall. The Darnall ward is in the top 10% of deprived areas in the country. The location of the centre was deliberately chosen to facilitate research projects in partnership with communities currently underserved by the health system. Therefore, utilising the AWRC within this study will further aim to breakdown health inequalities and ensure patients across all socioeconomic backgrounds can access the facilities and resources.

Is the primary focus of your study related to Funding Round Topic 4?
No

Benefit to Patients and the Public (150 words max)

Study findings will be disseminated through publication, presentations and public websites (Pancreatic Cancer and Mesothelioma UK). Funding acquisition (Cancer Research UK Clinical Trial Award) for a larger randomised controlled trial (RCT) in the year following this feasibility study.

An RCT over 3 years will assess the combination of exercise and immunotherapy in the first line palliative treatment setting in patients with advanced mesothelioma and in the maintenance setting in patients who've achieved a radiological response or stable disease following first line palliative chemotherapy for pancreatic cancer. Primary endpoint will be progression-free survival, with overall survival and objective radiological response rates as secondary endpoints.

Over the next 2 years, plan to implement exercise into clinical practice in Oncology. This would include prescribed exercise doses alongside immunotherapy and NHS funded exercise interventions.

This would mean improved radiological responses, longer duration of responses and improved progression-free and overall survival in these advanced cancers.

Section 4 - Recruitment and Participation
Recruitment

Please describe the recruitment setting for your study (for example, GP practices, secondary care, national screening databases) and how participants will be identified and approached. (150 words max)
Patients receiving first-line palliative immunotherapy for mesothelioma (n=15) or chemotherapy for pancreatic cancer (n=15) will be recruited from Weston Park Hospital covering the population across South Yorkshire. Trial investigators include Oncology clinicians managing advanced mesothelioma and pancreatic cancer. They will review clinic lists, identify and approach patients for study inclusion.

Total number of people to be recruited to study
30

Proportion of people to be recruited from Yorkshire (%)
100

Demographic data
Collection and reporting of demographic data

If your application is successful, do you confirm that you will collect, or endeavour to collect, and report the demographic data mentioned above for the study?

Yes

If you foresee any challenges in collecting the demographic data in line with the methods set out in the Demographic questions, please give details. (150 words max)
We do not foresee any challenges in collecting the demographic data in line with the methods set out in the Demographic questions.

Ethnic diversity
What is the expected ethnicity breakdown for those recruited to your study, as a percentage.

White - Pancreatic cancer (89%) Mesothelioma (94%)

Mixed or Multiple ethnic groups -Pancreatic cancer (0.4%) Mesothelioma (unknown)

Asian or Asian British - Pancreatic cancer (2.1%) Mesothelioma (0%)

Black, Black British, Caribbean or African - Pancreatic cancer (1.8%) Mesothelioma (0%)

Other ethnic groups - (including unknown) Pancreatic cancer (6.7%) Mesothelioma (6%)

Where the expected breakdown given does not align with the breakdown in the wider related population, please provide rationale for why this is different. (150 words max)
N/A

Please summarise any evidence and assumptions used to calculate the numbers reported above. (250 words max)

Ethnicity breakdowns for pancreatic cancer and mesothelioma were calculated from data collected from the British Journal of Cancer paper 'Differences in cancer incidence by broad ethnic group in England, 2013-2017' (1).

(1) https://www.nature.com/articles/s41416-022-01718-5/tables/1

Socioeconomic diversity
What is the expected deprivation breakdown (by quintile) for those recruited to your study, as a percentage.

1. Most deprived -Pancreatic cancer (17.1%) Mesothelioma (14.3%)

2. Pancreatic cancer (18.8%) Mesothelioma (18.3%)

3. Pancreatic cancer (20.9%) Mesothelioma (21.2%)

4. Pancreatic Cancer (22.2%) Mesothelioma (24.2%)

5. Least deprived - Pancreatic Cancer (21.0%) Mesothelioma (22.0%)

Where the expected breakdown given does not align with the breakdown in the wider related population, please provide rationale for why this is different. (150 words max)
N/A

Please summarise any evidence and assumptions used to calculate the numbers reported above. (250 words max)

The numbers were calculated above using pancreatic cancer incidence rates by deprivation statistics from 2020 from Cancer Research UK (1,2).

Pancreatic cancer incidence statistics | Cancer Research UK [Internet]. [cited 2024 Feb 16]. Available from: https://www.cancerresearchuk.org/health- professional/cancer-statistics/statistics-by-cancer-type/pancreatic-cancer/incidence#heading-Six

inc_dep_master_data_table_i17.xlsx [Internet]. [cited 2024 Feb 16]. Available from: https://view.officeapps.live.com/op/view.aspx?src=https%3A%2F%2Fwww.cancerresearchuk.org%2Fsites%2Fdefault%2Ffiles%2Finc_dep_master_data_table_i17.xlsx&wdOrigin=BROWSELINK

Age and biological sex
What is the expected biological sex breakdown by age group for those involved in your study, as a percentage.

Biological females

0 - 4	0	25 - 29	0	50 - 54	2	75 - 79	7
5 - 9	0	30 - 34	0	55 - 59	2	80-84	2
10 - 14	0	35 - 39	0	60 - 64	3	85 - 89	0
15 - 19	0	40 - 44	0	65 - 69	9	90+	0
20 - 24	0	45 - 49	1	70 - 74	15		

Biological males

0 - 4	0	25 - 29	0	50 - 54	2	75 - 79	12
5 - 9	0	30 - 34	0	55 - 59	3	80-84	2
10 - 14	0	35 - 39	0	60 - 64	5	85 - 89	0
15 - 19	0	40 - 44	1	65 - 69	14	90+	0
20-24	0	45-49	1	70-74	18		

Where the expected breakdown given does not align with the breakdown in the wider related population, please provide rationale for why this is different. (150 words max)

We have adjusted the numbers to more accurately reflect the population that are affected by pancreatic cancer and mesothelioma AND are fit enough and eligible to receive palliative treatment in the form of chemotherapy or immunotherapy. The wider population affected by these cancers include greater proportions of those 75 years and above including those above 90. Realisitically, in practice, we offer very few patients over the age of 90 systemic anti-cancer treatment, therefore numbers have been adjusted accordingly.

Please summarise any evidence and assumptions used to calculate the numbers reported above. (250 words max)

Using the Pancreatic Cancer and Mesothelioma UK incidence by age statistics from Cancer Research UK (1,2), we have calculated the above numbers. Given the higher incidence of both cancers in the advanced age population (70 years and above), similar distribution across age ranges is seen with both cancer groups.

Mesothelioma statistics | Cancer Research UK [Internet]. [cited 2024 Feb 1]. Available from: https://www.cancerresearchuk.org/health-professional/cancer- statistics/statistics-by-cancer-type/mesothelioma#heading-Two.
Pancreatic cancer statistics - Pancreatic Cancer UK [Internet]. [cited 2024 Feb 1]. Available from: https://www.pancreaticcancer.org.uk/what-we-do/media- centre/pancreatic-cancer-statistics/

Location of participation
What is the expected local authority breakdown for those involved in your study, as a percentage?

Barnsley	17	Leeds	0
Bradford	0	North Yorkshire	0
Calderdale	0	Rotherham	19
Doncaster	22	Sheffield	42
East Riding of Yorkshire	0	Wakefield	0
Kingston upon Hull	0	York	0
Kirklees	0	Other	Unchecked

Where will people predominantly take part in the intervention?
At patients' homes and the Advanced Wellbeing Research Centre, Sheffield Hallam University

Further information – demographic data. (250 words max)

All patients with advanced pancreatic cancer in South Yorkshire undergoing chemotherapy are treated at Weston Park Hospital. Oncology clinicians treating advanced mesothelioma see patients at Weston Park Hospital along with district general hospitals across South Yorkshire. Major conurbations of South Yorkshire being Barnsley, Doncaster, Rotherham and Sheffield. Therefore, local authority breakdown was calculated using population statistics from South Yorkshire (1).

(1) https://www.yhcouncils.org.uk/members/south-yorkshire/#:~:text=Population%3A%201%2C405%2C000%20Major%20conurbations%3A,Barnsley%2C%20Doncaster%2C%20Rotherham%20and%20Sheffield

Section 5 - Patient and Public Involvement and Engagement
Involvement and engagement of patients and the public

Members of the research team have attended a PPI workshop along with working with experienced members of cancer focus groups to review study documents and make sure we can listen, make improvements and see whether patients are on board with what we hope to gain from this trial.

We have had multiple discussions with dedicated PPI panels at the Mesothelioma UK Research Centre and Pancreatic Cancer UK focus group including outlining background, aims, methodology, impact and translational endpoints. The trial has received very positive feedback from cancer patients and their families. The potential of making immunotherapy beneficial for patients with pancreatic cancer was very well received. Mesothelioma patients attributed being physically active to their own good immunotherapy outcomes.

PPI has helped shaped the design of this trial with the inclusion of resistance training alongside aerobic exercise. PPI members will be included in our Trial Management Group to help study delivery.

Is your proposal a clinical trial?
Yes

Who is/are the study sponsor(s)?
Sheffield Teaching Hospitals NHS Foundation Trust

What criteria will be used to stop the study if necessary? (200 words max)

Criteria for electively stopping the trial or other research prematurely include failure to recruit and safety concerns with exercise intervention.

If your study has a feasibility or pilot stage ahead of a full trial what are your proposed stop/go criteria between the feasibility stage and the full trial? (250 words max)

This funding application is solely for a feasibility study assessing the practicalities of an aerobic exercise and resistance training programme in patients undergoing palliative treated for advanced pancreatic cancer and mesothelioma. As long as the criteria to stop the study prematurely (see above) is not reached, this study will continue until the last participant has completed their 12-week exercise programme.

Section 7 - Project Costings

Project costings

Budget heading		Year 1	Year 2	Year 3	Year 4
Salary					
Research Assistant	Cost	£11,141.00	£2,823.00	£0.00	£0.00
Prof J Wadsley	Cost	£8,425.00	£2,106.00	£0.00	£0.00
Clinical Trials Assistant	Cost	£7,098.00	£0.00	£0.00	£0.00
Dr B Hapuarachi	Cost	£8,001.00	£8,001.00	£0.00	£0.00
Salary Total	*Cost*	*£34,665.00*	*£12,930.00*	*£0.00*	*£0.00*
Non-salary					
Generable consumable cost to cover cell culture, plastics and reagents	Cost	£9,000.00	£9,000.00	£0.00	£0.00

Budget heading		Year 1	Year 2	Year 3	Year 4
Flow cytometry antibodies	Cost	£4,750.00	£4,750.00	£0.00	£0.00
PCR	Cost	£200.00	£175.00	£0.00	£0.00
RNA extraction kits	Cost	£4,000.00	£4,000.00	£0.00	£0.00
Additional kits to assess NK cell and T cell activation	Cost	£2,500.00	£2,500.00	£0.00	£0.00
Nanostring	Cost	£4,210.00	£4,211.00	£0.00	£0.00
PPI activity	Cost	£1,000.00	£1,000.00	£0.00	£0.00
Travel	Cost	£750.00	£750.00	£0.00	£0.00
Blood sampling - transport	Cost	£973.00	£242.00	£0.00	£0.00

Blood sampling - consumables	Cost	£479.00	£119.00	£0.00	£0.00
Archiving	Cost	£0.00	£750.00	£0.00	£0.00
Consumables / Printing - PIS / postage	Cost	£75.00	£75.00	£0.00	£0.00
Imaging - standard of care scans used - RECIST reporting	Cost	£4,050.00	£0.00	£0.00	£0.00
Flow cytometry (Aurora) - 30 patients x 8 time points x 0.5 = 120 hours at a cost of £55 per hour	Cost	£6,600.00	£0.00	£0.00	£0.00
Luminex / flow cytometer for multiplex / cytokine bead arrays - 240 samples	Cost	£5,000.00	£0.00	£0.00	£0.00
Non-salary Total	*Cost*	*£43,587.00*	*£27,572.00*	*£0.00*	*£0.00*
Grand Total	Cost	£78,252.00	£40,502.00	£0.00	£0.00

Budget heading		Total
Salary		
Research Assistant	Cost	£13,964.00
Prof J Wadsley	Cost	£10,531.00
Clinical Trials Assistant	Cost	£7,098.00
Dr B Hapuarachi	Cost	£16,002.00
Salary Total	*Cost*	£47,595.00
Non-salary		
Generable consumable cost to cover cell culture, plastics and reagents	Cost	£18,000.00

Flow cytometry antibodies	Cost	£9,500.00
PCR	Cost	£375.00
RNA extraction kits	Cost	£8,000.00
Additional kits to assess NK cell and T cell activation	Cost	£5,000.00
Nanostring	Cost	£8,421.00
PPI activity	Cost	£2,000.00
Travel	Cost	£1,500.00
Blood sampling - transport	Cost	£1,215.00
Blood sampling - consumables	Cost	£598.00
Archiving	Cost	£750.00

Budget heading		Total
Consumables / Printing - PIS / postage	Cost	£150.00
Imaging - standard of care scans used - RECIST reporting	Cost	£4,050.00
Flow cytometry (Aurora) - 30 patients x 8 time points x 0.5 = 120 hours at a cost of £55 per hour	Cost	£6,600.00
Luminex / flow cytometer for multiplex / cytokine bead arrays - 240 samples	Cost	£5,000.00
Non-salary Total	*Cost*	£71,159.00
Grand Total	Cost	£118,754.00

Total support requested in preliminary application
£118,754.00

Justification for difference in costs (300 words max)

The below costs have been added subsequently to the preliminary application, for more in-depth analysis of immune activation with exercise in these advanced cancer patients, which would further support the combination of exercise and immunotherapy in a randomised controlled trial.

NanoString - £8421
This will cover the cost of comprehensive profiling of the immune response to exercise training. Costs for 24 samples allowing analysis at different time points.

Additional kits to assess NK cell and T cell activation - £5000
Covers cost of running 60 samples to allow comparison of pre- and post- exercise samples across 30 participants. This will cover 2 x NK cell isolation kits and NK cell Activation/isolation kits along with Human Interferon gamma ELISA kits The costs also cover running the samples on the Cytek Flow Cytometer (£6600) and performing cytokine bead arrays (£5000).
Furthermore, the costs RECIST 1.1 reporting (£4050), blood transport and sampling (£1215 and £598 respectively) as well as salary costs have all been adjusted to current rates given by Sheffield Teaching Hospitals and Sheffield Hallam University finance departments.

The salary costs will also cover the costs of the Chief Investigator (Dr Hapuarachi) from October 2025 until study completion at 40% FTE (£16,002). Dr Hapuarachi is currently on a fully-funded PhD fellowship which will end in October 2025, when he will return to clinical training under Sheffield Teaching Hospitals. We therefore, request further funding for him to continue as Chief Investigator for the study to perform both clinical and non-clinical aspects of this study from October 2025 to May 2026.

Further discrepancies in cost differences are due to the inclusion of DA salary costs and overheads within the preliminary application costings, which have not been included in this full application as these would not be funded by the YCR charity.

Section 8 - Team and Project Details

Project team (100 words max)

Professor Muthana provides expertise on reprogramming tumours to become more immune responsive. Professors Danson and Wadsley provide clinical expertise in mesothelioma and pancreatic cancer. Dr Hapuarachi, oncology registrar, has performed pre-clinical studies exploring exercise on immunity in these cancers.
Dr Dalton and Dr Humphreys have expertise in studying exercise and designing/delivering exercise interventions for cancer patients.
Together we have >20 years' experience in designing and leading clinical trials. Weston Park Hospital has consistently been in the top 5 highest UK recruiters for pancreatic cancer trials. Exercise interventions will take place at the AWRC, a world-class research centre with state-of-the-art facilities.

Equity, Diversity and Inclusion (100 words max)
We are a diverse team from different ethnic backgrounds. Professor Muthana is School Director of One University (https://www.sheffield.ac.uk/vision/our- pillars/one-university) with involvement in cancer community projects (https://www.1in4spsg.org/#:~:text=The%20Sheffield%20Prostate%20cancer%20Support,Prostate%20Cancer%20in%20their%20lifetime).

In 2021, Professor Danson created/chaired a national conference session (NCRI) on Equality, Diversity and Inclusion in the research workforce. Dr Dalton led the Athena Swan gender equality submissions in 2015 and 2019 renewing their Silver award both times.
AWRC is partnered with Darnall Wellbeing, training/funding researchers from the local community helping recruit a more diverse group of participants. These measures demonstrate how our team actively strive for EDI whilst following NIHR-INCLUDE guidance.
Rese

Have you sought advice from any relevant National Groups or Initiatives?
Yes

If so provide details.

The study has been discussed with NIHR Research Design Service and presented to a panel of specialists and lay members at an NCRI proposal guidance meeting with advice taken on board in all aspects of our study including construction of our study protocol. We have also subsequently discussed the study with advisors and set up a pancreatic cancer focus group via the Pancreatic Cancer UK Research Involvement Network and a dedicated patient and public involvement panel via Mesothelioma UK Research Centre.

Section 10 - Public Engagement and Charity Promotion Opportunities
Please outline what approaches or suggestions you and your team may have to raise brand awareness including any fundraising activity. (250 words max)

We will utilise media teams from the University of Sheffield, Sheffield Teaching Hospitals and Sheffield Hallam University to develop our own webpage to disseminate this research and ensure YCR branding is incorporated in all promotions and research outputs including publications and conferences. We will do this regularly throughout the terms of the project and where possible, we will engage with local media outlets, including Sheffield Star and BBC Radio Sheffield.

Our research explores exercise to improve cancer treatments, which is a particular interest of the YCR More Life to Live scheme. Therefore, as this research will be discussed in a wide variety of research communications including Oncology Clinician and immuno-oncology conferences, AWRC / exercise seminars as well as across already established patient groups from Pancreatic Cancer UK and Mesothelioma UK Research Centre, we will also ensure YCR brand awareness, highlighting all YCR relevant research objectives. We will consult with these groups to discuss fundraising opportunities to support YCR across the country.

If this study is successful, we will promote the benefits of exercise in cancer publicly with outreach work to inform these benefits to all communities and raise YCR brand awareness.

Please complete these sections in Calibri font size 10 with 1.5 line spacing.

The combined project background and detailed project plan should total no more than **2,500 words** plus up to 5 figures/tables

Project Title

Exercise to Boost Immunity in advanced Cancer: feasibility of combined aerobic Exercise and resistance training for Patients with advanced mesothelioma and pancreatic cancer (BICEP)

Project Background

Please ensure that your project background includes a description of why the research is needed.

Immunotherapies, such as immune checkpoint inhibitors (ICIs) have greatly improved the survival outcomes across multiple different cancers. ICIs facilitate cancer cell recognition and destruction by immune cells by inhibiting pathways they use to evade the immune system. Although ICIs have demonstrated significant benefits in cancer, fewer than 40% of patients derive significant benefit from ICIs(1,2). This highlights the need to better understand the mechanisms of immunotherapy resistance and how to overcome this to improve responsiveness.

Pancreatic cancer is the 10th most common cancer in the UK but is the 5th most common cause of cancer-related death, with a 5-year survival rate of <7%(3). Pancreatic cancer is a 'cold tumour' due to the lack of immune cells within the tumour microenvironment and a less favourable immune profile demonstrating limited response to anti-cancer treatment(4,5). ICIs have not demonstrated effectiveness in pancreatic cancer and the prognosis of metastatic pancreatic cancer remains poor with a median overall survival of 11.1 months with optimal chemotherapy and a 30% response rate(6). Furthermore, fatigue, neutropenic sepsis, weight loss and other chemotherapy-mediated toxicities significantly reduce the quality of life of the months they have remaining.

Mesothelioma accounts for less than 1% of all new UK cancer cases however incidence has increased by 57% over the past 30 years, due to occupational asbestos exposure(7). Mesothelioma could be classed as a 'hot tumour' due to the presence of immunogenic cells within the tumour microenvironment. Dual ICIs with nivolumab and ipilumumab have some success in the treatment of advanced mesothelioma although only 40% respond with a median overall survival of 18.1 months(8). This is a relatively modest benefit when compared to ICI response to a classically hot tumour such as melanoma, where the overall survival surpasses 60 months with a 60% response rate(9).

There is a clear unmet need in both these groups of patients to improve overall response and survival outcomes as well as quality of life. Exercise would be a simple, cost-effective method to utilise in these patients with its known health benefits and potential impact on cancer/treatment related side effects and quality of life enhancement(2,3).

Exercise benefits patients with cancer including improving overall survival, reducing cancer-related and treatment-induced fatigue and reducing cancer recurrence(4,10). There is growing evidence that exercise can modulate local tumour and systemic immunity in patients with cancer, skewing the immune profile to favour anti-tumour activity and to support ICIs' mechanisms of action. Therefore, exercise could be crucial in improving responses and survival outcomes in patients undergoing immunotherapy, such as advanced mesothelioma, as well as expanding the spectrum of cancers that can obtain benefit from immunotherapy, such as pancreatic cancer. This research focuses on these two cancer groups.

There is currently no tailored exercise advice for patients with advanced cancer. It would be prudent to define the exercise event to provide a reproducible, standardised intervention in cancer care whilst also tailoring the exercise activities to the individual's needs and capabilities(4). This feasibility study will aim to define this exercise and demonstrate that exercise boosts immunity in patients with difficult-to-treat cancers. Once we can establish feasibility of exercise training in advanced mesothelioma and pancreatic cancer patients, this will allow larger studies exploring the efficacy of exercise as an adjunct to immunotherapy in these patients.

Full Project Description

Please ensure that your project description includes:

1. A description of how your research will address the need identified.
2. A project plan.
3. Primary and secondary outcomes.
4. Project milestones and clear delivery timetable.
5. Cost justifications.

Relevant power calculations, if your work has statistical analyses.

This research is vital to establish acceptable exercise regimes for patients with advanced mesothelioma and pancreatic cancer who can be significantly limited by respiratory difficulties, cancer cachexia and frailty. This study will allow patients access to supervised exercise activities with exercise specialists to help overcome these barriers. With already known general health benefits, the research will feed into larger randomised controlled trials comparing the combination of immunotherapy and exercise interventions to standards of care to determine the role exercise can have to improve immunotherapy responsiveness in these cancers and bridge the gap between hot and cold tumour response to immunotherapy. This research aims to improve the physical and mental wellbeing, treatment response and survival outcomes of patients with these poor prognostic cancers.

Project Plan

Design:

Feasibility study

Setting:

Recruitment will include patients (n=30) from South Yorkshire, receiving first-line immunotherapy for advanced metastatic mesothelioma (n=15) and patients receiving first-line chemotherapy for advanced pancreatic cancer (n=15). Patients will attend the Advanced Wellbeing Research Centre (AWRC) for a supervised exercise session once a week to include aerobic exercise and resistance training along with an unsupervised weekly exercise session for 3 months.

Participants:

Inclusion criteria	Exclusion Criteria
Patients about to start or undergoing first-line palliative immunotherapy for advanced, unresectable or metastatic mesothelioma.	More than one primary cancer
Patients about to start or undergoing first-line palliative chemotherapy for advanced, unresectable or metastatic pancreatic cancer.	Uncontrolled brain or bone metastases
Age over 18 years old	Patients who have progressed on first-line palliative immunotherapy for advanced mesothelioma.
Histological or cytological diagnosis of mesothelioma or pancreatic cancer.	Patients who have progressed on first-line palliative chemotherapy for advanced pancreatic cancer
ECOG Performance status 0-1 (to be assessed by clinician)	Unable to provide informed consent
Ability to provide informed consent	

Sample size:

Sample size for this feasibility study was chosen on agreement within the research team and under advice from a medical statistician accounting for possible variability around feasibility measures, practicalities of recruitment and adequacy to determine feasibility of the intervention in these cancer cohorts. 15 patients with mesothelioma and 15 patients with pancreatic cancer.

With an incidence of 15.4/100,000 for women and 20.2/100,000 for men, an estimated 250 patients per year are expected to present with pancreatic cancer in South Yorkshire (population 1.4 million). With up to 70% of patients deemed too unwell for any intervention and 10% of patients presenting early enough for curative surgery. With 2718 new case of mesothelioma in the UK per year, approximately 60 new cases are expected in South Yorkshire. Recruiting 15 patients from each group (6% of new pancreatic cancer and 25% of new mesothelioma patients in South Yorkshire) was agreed to be acceptable and practical.

<u>Table of Objectives and Outcome Measures</u>

All objectives relate to patients with advanced mesothelioma or pancreatic cancer undergoing treatment.

Objectives	**Outcome Measures**	**Timepoint Measured At**
Primary		
Determine the feasibility of exercise during treatment in patients with advanced mesothelioma or pancreatic cancer.	Adherence rate: The ratio of the number of exercise sessions performed to the number of exercise sessions planned	At cessation or completion of the study
Secondary		
Determine the safety of exercise in these patients.	The number of directly related adverse events.	Throughout the study
Determine the acceptability of exercise in these patients.	Acceptability rate: the ratio of those recruited to the trial to those approached as eligible.	At cessation or completion of the study
	Attrition rate: the ratio of dropouts from the trial to those enrolled initially.	At cessation or completion of the study
Determine the effect of exercise on immune cell activation in these patients.	Peripheral blood mononuclear cells (PBMC) in the blood. Immune cell activation will be determined by an increased in effector (Natural Killer cells / CD8 T cells) and a decrease in immunosuppressive cells (regulatory T cells) using flow cytometry.	Baseline, then 4-weekly pre- and post- exercise interventions for 12 weeks and at completion.
Determine the effect of exercise on cytokines/myokines in the blood, which are known to promote immune system activity	Cytokines/myokines in the blood (e.g. TNFα, IFNγ, IL-4, 5, 6, 7 and 15) using flow cytometry, RNA extraction/PCR and NanoString immune profiling.	Baseline, then 4-weekly pre- and post- exercise interventions for 12 weeks and then at completion.
Determine the effect of exercise on radiological response or progression.	Evidence of radiological response or progression as per RECIST 1.1 reporting of CT scans	Baseline and 12 weeks
Determine the effect of exercise on lung function.	Lung function as per results of lung function tests	Baseline and completion

Intervention:

The following steps outline what will happen for each participant.

Recruitment
- Discussion of the study with a member of the research team, including review of screening procedures to ensure patient fits the inclusion criteria and to ensure there are no individual unforeseen risks with undertaking exercise activities
- Review and sign an informed written consent form

Baseline investigations
- Blood samples will be collected at baseline
- Baseline Computed Tomography (CT) scans (standard of care) will be reported as per RECIST 1.1 criteria
- Lung function tests to assess baseline functional capacities

Exercise (during immunotherapy or chemotherapy)
- One weekly supervised and one weekly unsupervised combined aerobic exercise and resistance training activities with bloods taken monthly for 3 months pre and post supervised exercise
- 3-month regimen

End of exercise investigations
- Standard of care CT scan to be done at completion of 3-month exercise regimen and reported as per RECIST 1.1 criteria and compared to baseline
- Lung function tests to assess functional capacities following completion of exercise training

End of study
- Following the end of exercise investigations, no further involvement in the study is required, no further study follow up.
- Patient will continue with standard of care management

Ongoing research
- Any of the study participants can keep in contact with the research team regarding further studies. We will directly contact participants if they would like any further information regarding any study publications or presentations.

Baseline information will be collected including age, sex, race/ethnicity, medical conditions and medications, normal level of physical activity, type and stage of cancer and information regarding previous cancer treatments. A full medical history including physical examination, baseline measurements of height and weight and general observations will be performed. Quality of life will be assess using EQ-5d questionnaires throughout the study.

Exercise activities: The exercise programme will include one supervised exercise session per week at the Advanced Wellbeing Research Centre and one unsupervised home-exercise session per week.

The following points describe the FITT (Frequency, Intensity, Type and Time) principles used to plan the exercise programme. All exercise activities will be tailored to the individual accounting for their needs and abilities.

Frequency: Twice weekly, one supervised and one unsupervised session a week for 3 months

Intensity: Moderate intensity aerobic interval exercise (performing at 60% maximum heart rate) combined with 1-3 sets of 6-12 Repetition Maximum (RM) resistance training

Type: Aerobic interval exercise (cycling) and Resistance training (3 exercises: chest press, biceps and leg curl)

Time: At 4 x 4 minute cycling at 60%, max HR with 3 minutes active recovery. 3 sets with 2 minutes per set – around 20 minutes resistance training

Participants will be given specialist advice regarding the unsupervised home-exercise session. Participants will be given wearable devices (Fitbits) to track their heart rate, monitor activity and sleep. Participants will be advised to wear this during their home exercise and continuously for 1 week at the beginning of the exercise programme and 1 week at the end. Participants will also have the option to wear the devices all the time. This will allow us to account for physical activity/ exercise outside of the study. These weekly sessions will not interfere with any of their cancer appointments or treatments. These sessions will last over a 3-month period.

Lung function tests will be performed at baseline and at the end of the study (at 3 months, or earlier if they leave the study sooner).

They will have computed tomography (CT) scans every 3 months as standard of care. No further scans are required for this study.

Safety assessment:

Trained specialists will supervise exercise at the AWRC. Any adverse event related to the exercise intervention will be clearly documented and reported to the research team. For any adverse events that occur during the unsupervised exercise session, participants will have a clear out-of-hours contact number to report these.

The risk of falling/ sustaining an accident/ over-exertion would be minimised during the supervised session when an exercise specialist with experience in working with people with cancer is present acknowledging individual patient limitations. Participants with cardiovascular, respiratory or musculoskeletal concerns will not be included.

There will be standardised exercise protocols in place that will be graded according to the patients' needs and abilities. This will then dictate advice on unsupervised exercise interventions. All exercise protocols have an associated risk assessment that has assessed and mitigated any risks associated with the exercise sessions specifically for people with cancer.

Subject withdrawal:

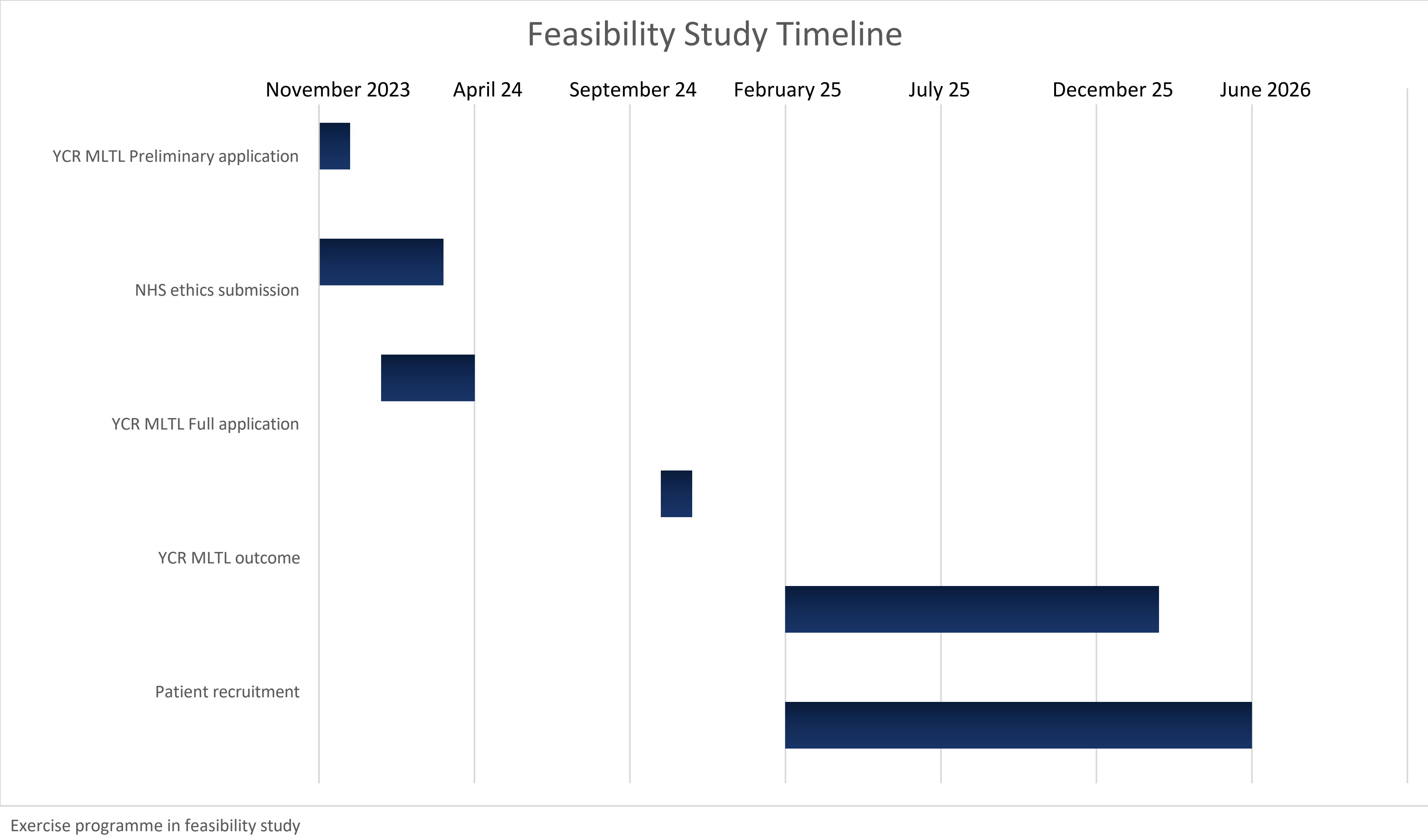
Criteria for electively stopping the trial or other research prematurely include failure to recruit and safety concerns with exercise intervention. Patients can withdraw at any point if they wish and continue with standard of care management.

Quality control:

Sheffield Teaching Hospitals has a responsibility to ensure all research within the Trust is conducted in accordance with the relevant legislation and guidelines. The Clinical Research and Innovation Office have standard operating procedures covering key activities, which will be included in the study Site File.

Timescale:

15 months (February 2025 – April 2026)


Feasibility Study Timeline
November 2023
April 24
September 24
February 25
July 25
December 25
June 2026
YCR MLTL Preliminary application
NHS ethics submission
YCR MLTL Full application
YCR MLTL outcome
Patient recruitment
Exercise programme in feasibility study

Cost justifications

Clinical Research Assistant (G6 SP23): £13964

Staff with expertise in exercise and working with people with cancer providing supervision for exercise interventions at the AWRC and tailoring individual plans for home-based activities.

Materials and consumables: £18000

Running costs of the project evenly distributed over the project duration including general lab biomaterials, plastics, culture medium, tissue culture supplements and all general lab chemicals over the 15 months.

Blood sampling consumables and transport £1813

For blood sampling performed throughout. Blood will be transported via courier to the University of Sheffield, where samples can be prepared and analysed within the laboratories.

PCR/RNA extraction kits £8375

To assess gene/ protein expression measuring immune activation secondary to exercise. Due to changes at the RNA level, we can then assess different cytokine expressions and analyse changes in immune cytokine profile with exercise.

NanoString £8421

For comprehensive profiling of the immune response to exercise training. Costs for 24 samples allowing analysis at different time points.

Flow Cytometry antibodies £9500

To assess changes in leucocyte populations with exercise training using flow cytometry. This will allow quantification of NK cells, CD4 T-cells, CD8 T-cells, B cells and monocytes and T cell activation.

Flow cytometry (£6600) and cytokine bead array (£5000)

To cover running samples for 30 patients

Kits to assess NK cell and T cell activation £5000

To run 60 samples to allow comparison of pre- and post- exercise samples across 30 participants and two NK cell isolation kits and NK cell Activation/isolation kits along with Human Interferon gamma ELISA kits.

PPI activities £2000

To work with patients and the public to shape our research, and engage with it. This is essential to help inform the direction of our project. This will cover transport, refreshments, member reimbursement for time/ electricity if virtual meetings and costs of any additional supplies required for activities (e.g. stationary).

Travel £1500

To reimburse travel for participants to and from the AWRC for their supervised exercise activities, which will take place weekly for 12 weeks for each participant.

RECIST reporting £4050

To report standard of care scans as per RECIST 1.1 criteria to standardise the classification of response on imaging.

STH printing and archiving £900

To cover study documentation including participant information sheets and information storage.

Clinical trials assistant and data manager £7098

Funding for staff to assist in patient recruitment, data collection, study monitoring and point of contact for participants.

Chief Investigator salary (40% FTE) £16002

Covers Dr Hapuarachi's salary as Chief Investigator from October 2025 (PhD completion) to May 2026 (study completion).

Clinical supervisor (NHS) salary £10531

Professor Wadsley will be clinical supervisor and pancreatic cancer expert for this study.

Future work

This study will confirm feasibility and explore the effect of defined exercise doses on immunity of patients with advanced pancreatic cancer and mesothelioma. Study findings will be disseminated through publication, presentations and public websites (Pancreatic Cancer and Mesothelioma UK). Funding acquisition (Cancer Research UK Clinical Trial Award) for a randomised controlled trial (RCT) in the year following this feasibility study.

An RCT over 3 years will assess the exercise-immunotherapy combination in the first-line palliative treatment setting in patients with advanced mesothelioma and in the maintenance setting in patients who have achieved a radiological response or stable disease following first-line palliative chemotherapy for pancreatic cancer.

Primary endpoint will be progression-free survival, with overall survival and objective radiological response rates as secondary endpoints.

Over the next 2 years, plan to implement exercise into clinical practice in Oncology. This would include prescribed exercise doses alongside immunotherapy and NHS funded exercise interventions. This would mean improved radiological responses, longer duration of responses and improved progression-free and overall survival in these advanced cancers.

References and supporting literature (please use Vancouver style and list each reference in numerical order.)

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2. Bird BH, Nally K, Ronan K, Clarke G, Amu S, Almeida AS, et al. Cancer Immunotherapy with Immune Checkpoint Inhibitors-Biomarkers of Response and Toxicity; Current Limitations and Future Promise. Diagnostics (Basel, Switzerland) [Internet]. 2022 Jan 1 [cited 2022 Dec 2];12(1). Available from: https://pubmed.ncbi.nlm.nih.gov/35054292/

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4. Gustafson MP, Wheatley-Guy CM, Rosenthal AC, Gastineau DA, Katsanis E, Johnson BD, et al. Exercise and the immune system: taking steps to improve responses to cancer immunotherapy. J Immunother Cancer [Internet]. 2021 Jul 2 [cited 2022 Dec 1];9(7):1872. Available from: /pmc/articles/PMC8256759/

5. Maleki Vareki S. High and low mutational burden tumors versus immunologically hot and cold tumors and response to immune checkpoint inhibitors. J Immunother cancer [Internet]. 2018 Dec 27 [cited 2022 Dec 2];6(1). Available from: https://pubmed.ncbi.nlm.nih.gov/30587233/

6. Conroy T, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, et al. FOLFIRINOX versus Gemcitabine for Metastatic Pancreatic Cancer. N Engl J Med [Internet]. 2011 May 12 [cited 2023 Jun 16];364(19):1817–25. Available from: https://www.nejm.org/doi/full/10.1056/NEJMoa1011923

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8. Baas P, Scherpereel A, Nowak AK, Fujimoto N, Peters S, Tsao AS, et al. First-line nivolumab plus ipilimumab in unresectable malignant pleural mesothelioma (CheckMate 743): a multicentre, randomised, open-label, phase 3 trial. Lancet [Internet]. 2021 Jan 30 [cited 2023 Jun 16];397(10272):375–86. Available from: http://www.thelancet.com/article/S0140673620327148/fulltext

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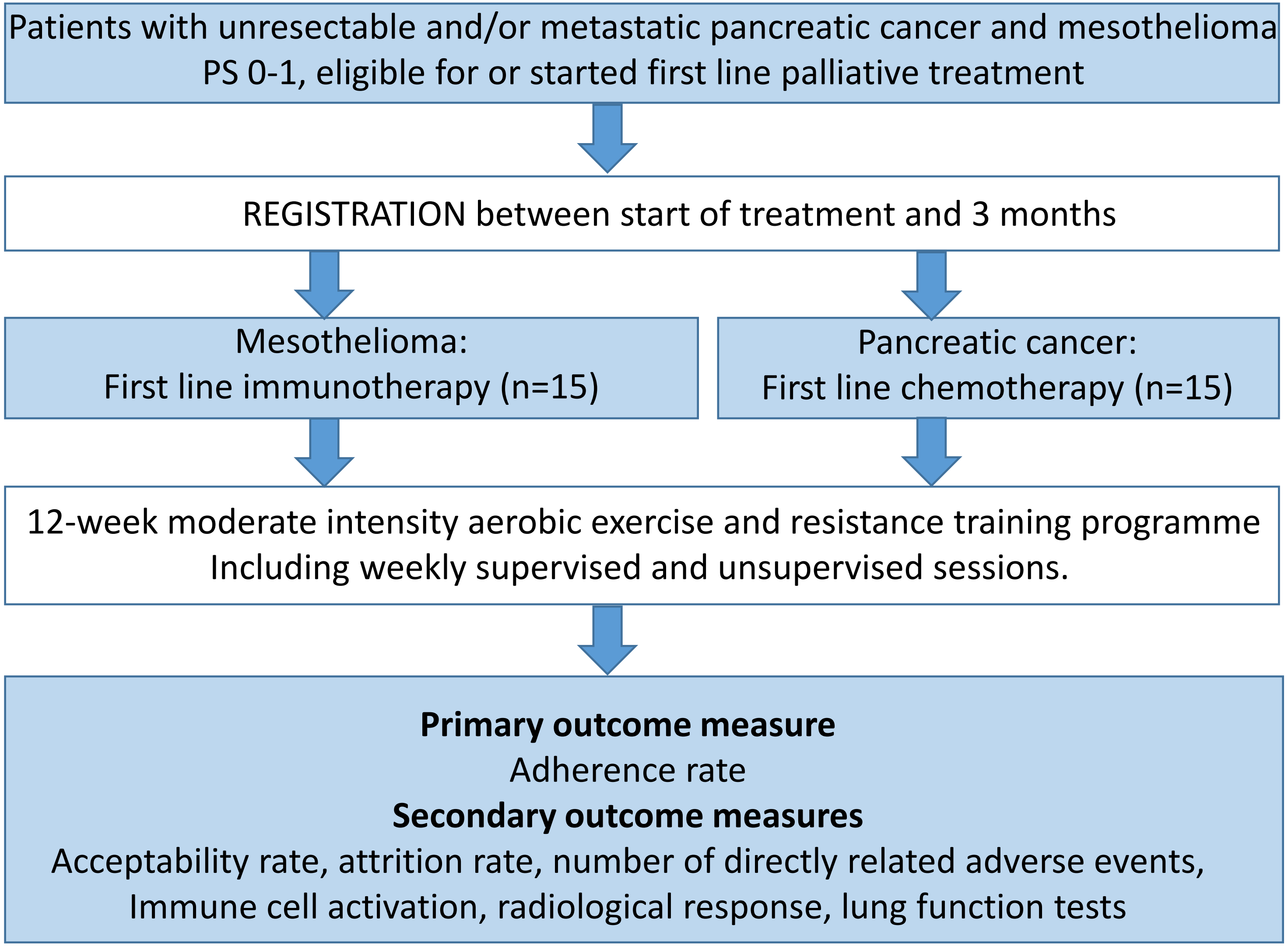
Appendix C: Trial flowchart



Appendix D – Schedule of Assessments

Visits	Screening	Baseline	Intervention Period											End of Trial
Scheduling Window	Week -4 to Week -2	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13
Eligibility Check	X														
Trial Discussion	X														
Informed Consent		X													
Data Collection[1]		X													
Physical Exam		X													
Height & weight		X													
Vitals		X	X	X	X	X	X	X	X	X	X	X	X	X	
Immune Profiling		X				X[2]				X[2]					X
Cytokines/ Myokines		X				X[2]				X[2]					X
CT Scans		X[3]													X[3]
Lung Function Tests		X													X
Exercise Intervention			X	X	X	X	X	X	X	X	X	X	X	X	

1 Age, sex, race/ethnicity, medical conditions and medications, normal level of physical activity, type and stage of cancer and information regarding previous cancer treatments

2 Done pre and post supervised exercise intervention

3 Done in line with standard of care.

Appendix E: Patient information sheet

Exercise to Boost Immunity in advanced Cancer: feasibility of combined aerobic Exercise and resistance training for Patients with advanced mesothelioma and pancreatic cancer (BICEP)

We are inviting you to take part in this research study looking into the role of exercise activity in cancer care. It's up to you whether you take part or not. Please read this information sheet which will outline why we are doing this research, and what it will involve. Feel free to talk this over with whomever you wish, including your doctor, family and friends before making a decision. Please do not hesitate to ask us any questions to clarify any part of the study or if you would like more information.

Why are you doing this study?

Immunotherapy is a type of cancer treatment that helps the body's immune system fight cancer. It works by helping immune cells recognise and attack cancer cells. Immunotherapy works well in certain cancers, however, it does not work in others. Exercise has been shown to improve general outcomes in everyone including cancer patients. Further research is required to give a better understanding on the role of exercise and how it might improve cancer care and the potential anti-cancer effects of exercise. We aim to add to this research and understand the role of exercise in improving a person's immune system to give immunotherapy the best chance of working.

Firstly, we need to ensure that we can find standard exercise activities that patients with cancer find acceptable, realistic and something that they can continue in the long term. Therefore, the main aim of this study is to explore this in patients with mesothelioma and pancreatic cancer. We have chosen these two very different cancers because they have different responses to immunotherapy.

Why have I been invited to take part?

This study is looking into exercise activities alongside the treatment that you will be receiving at Weston Park Hospital, Sheffield. You have been chosen because we think you are fit enough to have anti-cancer treatment and will be receiving either immunotherapy or chemotherapy for either your mesothelioma or pancreatic cancer.

What does the study involve?

If you agree to participate in the study, you will attend for the following visits. More information is provided later in this information sheet on what individual procedures involve:

Screening Visit

1) Discussion of the study with a member of the research team.
2) Screening procedure to ensure you fit certain criteria to take part, as well as to ensure there are no unforeseen risks with you carrying out regular exercise activities.

Baseline Visit

1) Review and sign an informed written consent form
2) Collecting baseline information including your age, sex, race/ethnicity, medical conditions and medications, normal level of physical activity, type and stage of cancer and information regarding previous cancer treatments.
3) Completing a full medical history including physical examination, baseline measurements of height and weight and general observations.
4) Taking blood tests for the study
5) Performing lung function tests which will assess different capacities in your lungs and how well they are working.
6) Computed Tomography (CT) Scans to assess the disease in your chest, abdomen, and pelvis.

Intervention Visits

1) Exercise activities: The exercise programme will include one weekly supervised exercise session at the Advanced Wellbeing Research Centre (AWRC) based at Olympic Legacy Park, 2 Old Hall Road, Sheffield, S9 3TU and one weekly unsupervised exercise session, at your own home. The supervised session will include cycling on an exercise bicycle with periods of rest with an exercise specialist present who will be monitoring your vital signs and tailoring your exercise to ensure the level at which you are exercising is appropriate and safe. The session will also include supervised resistance training. You will then be given further advice regarding the unsupervised exercise session from the specialist. You will be provided with a wearable device (Fitbit) which can monitor and record your heart rate and step count during you home exercise activity as well as periods of activity during the week. Weekly supervised sessions will be arranged so that they do not interfere with any of your cancer appointments or treatments. Each supervised session will last around 30-45 minutes. These sessions will last over a 12 week period.
2) Performing lung function tests at 4-weekly intervals which will assess different capacities in your lungs and how well they are working.
3) Taking blood tests for the study at 4-weekly intervals before and immediately after planned supervised exercise activities.

End of Study Visit

1) Performing lung function tests which will assess different capacities in your lungs and how well they are working.
2) Taking blood tests for the study
3) CT Scans to assess the disease in your chest, abdomen, and pelvis.

Do I have to take part in the study?

No. It is entirely up to you whether you decide to take part in the study or not and it will not affect any aspects of your standard cancer treatment. If you do decide to participate, then you will be asked to sign an informed consent form. Please keep the information sheet for your records.

At any time, including after signing the consent form, you can stop participating and withdraw from the study and this will not negatively affect your cancer treatment in any way. You do not need to provide any reason for your withdrawal.

What are the possible benefits of taking part?

The specific anti-cancer benefits of exercise against your cancer type are largely unknown. However, exercise has been shown to improve overall health and fitness and benefit other cancers. This study offers support from an exercise specialist whilst undergoing supervised exercise. Exercise could also potentially help with cancer-related and treatment-related side effects like tiredness as well as help support mental health. Parking is free at the AWRC and there will be some reimbursement for reasonable costs including transportation to the AWRC.

What are the possible disadvantages and risks of taking part?

If you have any medical conditions, which would mean moderate intensity exercise would be risky, please do not join this study. These include, but are not limited to, angina, recent heart attack or any other heart conditions, recent stroke, recent bone fracture or central nervous system disorders. As previously explained, exercise is generally a recommended and safe activity but possible risks could include but not limited to musculoskeletal injury and low blood sugar levels in diabetic patients.

During your participation, you will have multiple additional blood tests. Possible side effects of needle insertion and drawing blood could include discomfort, minor bleeding and small risk of infection around the needle insertion site.

You will also have lung function tests, which will test how well your lungs are working. These tests are very safe. Some minor symptoms you may feel include dizziness, short of breath or having a cough. These symptoms are generally mild and do not last long.

CT Scans use ionising radiation to form images of your body and provide your doctor with other clinical information. If you take part in this study, you will not have any more scans than you would if you didn’t take part. Ionising radiation can cause cell damage that may, after many years or decades, turn cancerous. The chances of this happening to you are the same whether you take part in this study or not.

Study Procedures

Blood Tests

Around 2 teaspoons of blood will be each time you have your blood taken. Your blood samples will then be analysed to look at changes specifically related to exercise and the potential effect on cancer and cancer treatment. Your blood will not be tested for any other reason without your consent. These bloods tests will be extra to those you will have as part of your routine care. Blood samples will then be stored in a laboratory at the University of Sheffield. Samples may be used for future research and learning.

Lung Function Tests

Lung functions tests will involve you breathing into a mouthpiece connecting tubing, breathing out as long, hard and fast as you can. This will take place once a month when you attend the AWRC for your supervised exercise session. We will look for changes in how well your lungs work before, during and after the exercise programme.

CT Scans

Whether you take part in this study or not, if you are receiving anti-cancer treatment for mesothelioma or pancreatic cancer, you will have CT scans every 3 months. CT scans involve x-ray techniques to assess the disease in your chest, abdomen, and pelvis. If you agree to take part in the study, you will have the same number of scans as if you were not in the study. The CT scans you will receive are part of your routine care.

Will my taking part in this project be kept confidential and what will happen to the data collected?

We will need to use information from you and from your medical records for this research project.

This information will include your name, NHS number, contact details and medical history. People will use this information to do the research or to check your records to make sure that the research is being done properly.

People who do not need to know who you are will not be able to see your name or contact details. Your data will be pseudonymised, this means it will have a code number instead.

We will keep all information about you safe and secure.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

Your data will be governed by the Data Protection Act 2018 and the UK General Data Protection Regulation (2018). All of the medical and personal information we collect along with blood sample analysis, CT scan reports and lung function tests will be kept confidential and only research team members, including our collaborators at the AWRC, will be able to access this information. Identifiable personal data will be kept in secure, confidential NHS systems.

You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have.

If you withdraw from the study, no further new information will be collected. Any data, including blood samples, that have previously been collected prior to your withdrawal may still be processed, with any data anonymised and used for the research unless you specifically request for your data and samples to be destroyed and not used in the research.

We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about you.

Where can I find out more about how my information is used?

You can find out more about how we use your information

- at www.hra.nhs.uk/information-about-patients/
- our leaflet available from www.hra.nhs.uk/patientdataandresearch

- by asking one of the research team
- by sending an email to sth.infogov@nhs.net, or
- by ringing us on [**phone number**].

GP involvement

If you agree to take part in the study, our research team will let your GP know

Who is organising the research?

This research is being organised by the sponsor, Sheffield Teaching Hospital NHS Foundation Trust. Where "we" is used in this information sheet this refers to the sponsor.

Who is funding the research?

This research is part of a PhD project funded by Sheffield Teaching Hospitals Charity.

Who has reviewed the study?

This study has been reviewed and given approval by the Health Research Authority, NHS Research Ethics Committee and Sheffield Teaching Hospitals Clinical Research & Innovation Office.

If I wish to complain about the way in which this study has been conducted?

If you have any cause to complain about any aspect of the way in which you have been approached or treated during this study, the normal NHS complaints mechanisms are available to you, you may have to pay for further legal action if required. You will not be compromised in any way because you have taken part in a research study. You can contact the complaints team directly –The Patient Advice and Liaison Service.

Email: sth.pals@nhs.net

Telephone: 0114 2712400

We would like to thank you very much for considering taking part in this study and we would be happy to answer any further questions you may have.

Appendix F: Lay Summary

Exercise to Boost Immunity in advanced Cancer: feasibility of combined aerobic Exercise and resistance training for Patients with advanced mesothelioma and pancreatic cancer (BICEP)

Background

Immunotherapy is a type of cancer treatment that helps your own immune system fight cancer. It works by helping immune cells in your body and in the tumour recognise and attack cancer cells. Immunotherapy can be an effective treatment for many different types of cancers, however there is still work to be done to achieve better and longer responses to immunotherapy. Not all cancer patients respond to immunotherapies as their tumours do not contain active immune cells.

What we aim to do

This research investigates whether exercise can boost the immune system in cancer patients and make immunotherapies work better. Exercise can improve general outcomes in cancer patients. Further research is required to give a better understanding on the role of exercise and how it might improve cancer care. We hope to add to this research and understand the role of exercise in optimising a person's immune system to give immunotherapy the best chance of working.

In the first instance, we need to ensure that we can find standard exercise activities that patients with cancer find acceptable, realistic and something that they can continue in the long term. We will explore this in patients with mesothelioma and pancreatic cancer. We have chosen these two cancers due to their varying responses to immunotherapy.

Study recruitment and design

The study will involve the treating doctors and the research team recruiting patients with mesothelioma and pancreatic cancer who are currently receiving immunotherapy or chemotherapy. It will look at what exercise regimes are realistic in these groups of patients.

The exercise programme will include one weekly supervised exercise session at the Advanced Wellbeing Research Centre and one weekly unsupervised exercise session. The supervised session will include cycling on an exercise bicycle with periods of rest with an exercise specialist present who will be monitoring vital signs (heart rate, oxygen levels etc.) and tailoring the exercise to ensure the level at which the patient is exercising is appropriate and safe. If a participant feels unwell during the exercise activity, then their wellbeing will be the most important consideration. The exercise specialist will stop the exercise and they will be able to review and if needs be, arrange for a doctor review. If the participant felt too unwell to take part in the exercise activity on any given day, then they should not attend the session and seek support from Weston Park Hospital if needed.

Further advice regarding the unsupervised exercise session from the specialist will also be given. These weekly sessions will be arranged so that they do not interfere with any of the patient's cancer appointments or treatments. One session will last around 30-45 minutes. These sessions will last over a 12 week period.

We will take blood samples from these patients at regular time points before and after exercise, as well as assessing the response of the cancer to treatment using medical imaging (computed tomography (CT) scans). The blood samples will then be analysed to look at changes specifically related to exercise and the potential effect on cancer and cancer treatment. We will also check how well the lungs are working using lung function tests at regular points during the study.

The study will be based in Sheffield, and we will recruit patients from the local area.

Patient and Public Involvement

We understand the importance of involving patients and their families at all stages in setting up and carrying out this study. Members of the research team have attended a patient and public involvement (PPI) workshop along with working with experienced members of cancer focus groups to make sure we can listen, make improvements, and see whether patients are on board with what we hope to gain from this study. We have already listened and learnt from the opinions of patients and their families in the dedicated PPI group at the Mesothelioma UK Research Centre, Sheffield, and plan to continue working with them as well as further PPI groups who have been affected by pancreatic cancer.

An important part of this study will be to hear directly from patients about how they feel about the exercise regime and whether it had affected their physical and mental health, and if so, in what way by asking patients to fill out questionnaires during the study.

What happens after the study?

We will use the information from this study to design a larger study to look into the effect of exercise on the immune system and the effect it has on how well immunotherapy works. We will keep all those involved updated on the status of the study and where we hope to publish the information.

Appendix G: GP letter

Weston Park Hospital,
Witham Road,
Sheffield,
S10 2SJ.

[GP Practice]
[GP Address]
[GP address]
[GP Postcode]

Exercise to Boost Immunity in advanced Cancer: feasibility of combined aerobic Exercise and resistance training for Patients with advanced mesothelioma and pancreatic cancer (BICEP)

Dear Dr [GP Name]

Regarding: [Patient Name, DOB, Address]

I am writing to inform you that [Patient Name] has consented to participate in a feasibility trial exploring the acceptability and tolerability of supervised and unsupervised exercise activities during their anti-cancer treatment for [mesothelioma or pancreatic cancer].

The supervised exercise activity will take place at the Advanced Wellbeing Research Centre based at the Olympic Legacy Park, Sheffield, once a week for 12 weeks, with each session lasting around thirty to forty-five minutes. Please see attached the study information sheet.

If you have any further questions about this study, you can contact us by email (sonal.hapuarachi@nhs.net).

Yours sincerely,

Dr Brindley Hapuarachi

Specialist Registrar in Medical Oncology, Weston Park Hospital, Sheffield

Clinical Research Fellow in Translational Oncology, University of Sheffield

Appendix H: Consent form

Consent Form

Exercise to Boost Immunity in advanced Cancer: feasibility of combined aerobic Exercise and resistance training for Patients with advanced mesothelioma and pancreatic cancer (BICEP)

Please initials the appropriate boxes	*Initials*
Taking Part in the Project	
I have read and understood the project information sheet Version ____ dated ______________ and the project has been fully explained to me.	
I have been given the opportunity to ask questions about the study.	
I agree to take part in the study. I understand that taking part in this study will include extra blood tests at regular time points, up to 24 moderate intensity interval aerobic exercise activities combined with strength training (12 supervised and 12 unsupervised) undertaken alongside anti-cancer treatment as well as lung function tests.	
I understand that my taking part is voluntary and that I can withdraw from the study at any time; I do not have to give any reasons for why I no longer want to take part and there will be no adverse consequences if I choose to withdraw.	
I agree to my GP being informed of my participation in the study.	
How my information will be used during and after the project	
I understand my personal details such as name, phone number, address and email address etc. will not be revealed to people outside the research team.	
I agree that my data can be stored and used for this study. I understand that this data will be held by Sheffield Teaching Hospitals NHS Foundation Trust and University of Sheffield in compliance with the UK General Data Protection Regulation (2018) and Data Protection Act 2018.	
I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from Sheffield Teaching Hospitals NHS Foundation Trust or from Regulatory Authorities where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records	
I understand and agree that other authorised researchers which are part of the research team will have access to this data only if they agree to preserve the confidentiality of the information as requested in this form.	
I understand and agree that other authorised researchers within the research team may use my pseudonymised data in publications, reports, web pages, and other research outputs, only if they agree to preserve the confidentiality of the information as requested in this form.	

The following points are OPTIONAL

Please initials the appropriate boxes	*Initials*	
	Yes	*No*
I give permission for the blood samples that I provide to be deposited in the University of Sheffield laboratory so it can be used for future research and learning		

Name of participant [printed] Signature Date

Name of Researcher [printed] Signature Date

Original to be kept In site file, 1 copy to be given to the patient, 1 copy to be kept In the medical notes.

Project contact details for further information:
Lead researcher: Dr Brindley Hapuarachi (sbhapuarachchi1@sheffield.ac.uk)
Supervisors: Dr Munitta Muthana (m.muthana@sheffield.ac.uk), Professor Sarah Danson (s.danson@sheffield.ac.uk) and Professor Jonathan Wadsley (jonathan.wadsley1@nhs.net).

Appendix I – Patient and public involvement activities

Appendix I illustrates the positive comments received from members of patient and public involvement (PPI) groups including helpful insights that were incorporated into the study protocol. PPI activities included research members attending PPI workshop at the University of Sheffield and multiple discussions and meeting with different groups of patients affected by pancreatic cancer and mesothelioma.